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VACCINATION AGAINST FOWL PLAGUE

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INTRODUCTION

An examination of the literature on fowl plague suggests that no very great success has hitherto attended the efforts of workers to devise a satisfactory technique of artificial immunisation. Such efforts have been mainly concentrated on attempts to evoke immunity by injections of virus-containing tissues, inactivated either by heat or by treatment with chemical reagents. Thus Jouan and Staub (1920) claimed to have immunised birds by the injection of virulent blood that had been heated at 46° to 47° C. for 72 hours, or at 60° C. for 45 minutes, but their claims could not be substantiated by Doyle (1927), Todd (1928) and others, even when multiple large doses of heated blood were employed. In a later communication Staub (1926) reported successful immunisation of birds with a formolised spleen vaccine, but again his results could not be confirmed by Doyle (1927) or Todd (1928), the former using as many as six gradually increasing doses of formolised spleen suspension. Concurrently with his failure to immunise fowls with formolised spleen suspensions, Todd succeeded in immunising birds by injecting a course of three doses of a vaccine prepared from liver tissue of infected fowls treated with carbolic acid and glycerine. A combination of the methods of Staub (1926) and Todd (1928) was apparently adopted in Egypt "on an experimental scale" about 1933 (Carpano, 1933) and this was the only method employed in the field until 1947.

More recently, sporadic attempts have been made to improve the antigenic quality of inactivated tissue vaccines either by alternative methods of inactivation or by adding adjuvants, such as aluminium hydroxide gels. Thus Levin and Lominski (1936) succeeded in immunising birds with virulent blood inactivated by exposure to X-rays, but stated that it was difficult to gauge the precise amount of radiation that was required. Under-radiated blood still killed the birds, whereas over-radiated preparations were without antigenic value. Schmidt *et al.*, in a series of publications (1934, 1935, 1936) recorded work with aluminium gel-adsorbed suspensions of virulent tissue (chiefly mouse-brain) which were rendered safe for injection by ageing at 0° to 4° C. or 20° C. or less satisfactorily by incubation at 37° C. They described their results as encouraging, but it will be evident that in vaccines prepared by their methods the tissue (mouse-brain) would contain too little virus and too much foreign species protein, and their results indicate that the method of inactivation by ageing lacked precision. An attempt by Schmidt and Oerskov

(1935) to make use of Frazer and Walsh's oil emulsion method was unsuccessful.

Attacking the problem from another angle, that of producing an apathogenic strain of the virus, Hallauer (1939) carried the virus through 50 serial passages in tissue culture, but with no appreciable reduction in the virulence of the strain.

The present investigation was planned with two objectives; the short-term one of improving, if possible, the quality of the inactivated vaccine at present in use in Egypt, and the long-term one of trying to produce an apathogenic variant with satisfactory antigenic characteristics. This note will deal with experiments carried out on the short-term project only. Regarding the long-term issue, it will be sufficient to state that up to the time of writing success has not attended the efforts that have been made to attenuate the virus by serial cultivation in chick embryos, pigeon embryos, or duck embryos, or by serial passage through mice, ducklings or squab-pigeons, but this side of the work is being continued.*

MATERIALS AND METHODS

Until quite recently only one local strain of fowl-plague virus was available for investigation. It is referred to in a previous note (Daubney and Mansi, 1948) as the "A" strain, which was recovered by Dr. Bishlawy in the neighbourhood of Alexandria in 1945 during an extensive outbreak of the disease on one of the Ministry's breeding farms. The strain is highly virulent and, if anything, its virulence was slightly exalted during the first 30 passages in the chick embryo. During the next 30 passages its virulence for fowls may have declined slightly, but after three serial passages in adult birds, the period of survival of inoculated birds was reduced from 72 to 48 hours.

For routine propagation of the strain, embryos of 10 to 12 days from eggs laid by susceptible fowls have been used (ten days when growing the virus for vaccine production). The normal method of inoculation has been intra-allantoic. During the first 18 passages the embryos died in from 36 to 48 hours, but at this stage the period of survival was shortened to about 22 hours under incubation at 38° C. and 24 to 26 hours at 36° C.: it has remained constant at this level.

Willstätter's aluminium gel when employed as an adjuvant, has

* This paper was prepared in March/April, 1948, and the statement that no success had attended our efforts to induce mutations of the virus was correct at the time of writing. Since then, however, one recently isolated strain of virus under serial passage in the pigeon embryo has thrown up a mutant that is completely apathogenic for domestic fowls, turkeys, and young pigeons and, at the same time, is highly antigenic in that it immunises solidly against our most virulent strain of fowl-plague virus.

This variant is apparently stable when returned to the chick embryo for further cultivation. It seems likely this strain will prove a most satisfactory immunising agent against the disease.

been prepared by Dr. Hilmy in accordance with the formula given by Sabin (1932). For the oily vaccines, the mineral oil first used was ordinary liquid paraffin B.P. Later it was replaced by a white mineral oil P40, prepared by the Shell Company.

The birds used in the tests have been cockerels of various breeds, mostly crossed European-Egyptian, of known history and almost all obtained from Barrage farm. At the start of the experiments the birds were from four to five and a half months old. They have remained generally healthy throughout the period and the losses sustained have been largely caused by fighting. Vaccinated birds awaiting immunity test have been held on one of the experimental farms together with a large number of susceptible birds. There has been no outbreak of fowl plague on this farm during the period covered by the experiments. In most cases immunised and control birds have received a subcutaneous inoculation of a small dose of virus, but in some of the trials resistance was tested by exposure to contact infection.

EXPERIMENTAL

Since a preliminary test had shown that chicks could be immunised by a course of three injection of 1 c.c. of a formolised suspension of infective embryos, membranes and fluids, it was decided to compare the efficacy of vaccine prepared from chick-embryo material with the standard Egyptian vaccines made from the tissues (liver and spleen) of infected birds and to test the value of adjuvants, such as aluminium hydroxide gels and mineral oils with killed acid-fast bacilli.

The adjuvant properties of the aluminium gel, first employed by Schmidt in his foot-and-mouth disease vaccine and later developed to a high state of efficacy by Waldmann and his colleagues, are now well known ; and this method has recently been applied to the production of inactivated vaccines against rinderpest (Curasson, 1947), and Newcastle disease (Traub, 1944 ; Brandly, *et al.*, 1946). The oily excipient (mineral oil with lanolin), as a vehicle for antigens first suggested by Le Moignic and Pinoy (1916). has lately attracted renewed attention, and the technique has been found effective in enhancing the efficacy of antigens as widely dissimilar as typhoid and dysentery bacilli, tissue proteins, toxins. malaria parasites, simple chemical compounds and filtrable viruses. This oily excipient was successfully used by the senior author in the preparation of a bovine pleuro-pneumonia vaccine (Daubney. 1937). The addition of killed acid-fast bacteria to the oily suspension results in a further increase and prolongation of the antigenic response in the cases of typhoid bacilli (Freund and Bonato, 1944). chemical compounds (Landsteiner and Chase, 1942), influenza virus (Friedewald. 1944), and *S. dysenteriae* (Halbert *et al.*, 1946).

Trials with pools of virulent allantoic fluid and extracts of embryonic tissues showed that the virus, suspended in buffer solutions ranging from *p*H 6·8 to *p*H 9·0, was readily adsorbed by an aluminium gel. Adsorption appeared to be more nearly complete at *p*H 6·8 than at *p*H 9·0, the titre of the supernatant in a centrifuged preparation being reduced from 1 c.c. × 10^{-6} m.i.d. to less than 1 c.c. × 10^{-3} in 24 hours at 4° C. at the neutral level, while at the more alkaline level the reduction was to less than 1 c.c. × 10^{-4} only. Incubation of the whole gel suspension at 38° C. for 24 hours did not completely inactivate either adsorbed or free virus and the adsorbed virus survived better in the *p*H 6·8 buffer. In the refrigerator at 4° C. the adsorbed virus survived periods of 27 days without any marked loss of titre.

Formolised and aluminium gel-adsorbed allantoic fluid, and tissue vaccines

The object of the first test (Batch I) was to compare the efficacy of plain formalised allantoic fluid with that of aluminium gel adsorbed preparations of allantoic fluid at *p*H 6·8 and *p*H 9·0, and with tissue vaccines prepared from livers and spleens of infected birds. Three chick-embryo vaccines were prepared from a common pool of allantoic fluid, but it will be observed that the plain vaccine contained approximately two and half times as much virus per c.c. as the vaccines incorporating gel. The liver and spleen vaccines were prepared by Dr. Bishlawy from the organs of adult birds, artificially infected with one of the three stock strains of virus and freshly dead or killed at the point of death. The strains were those referred to in a previous note (Daubney and Mansi, 1948) as "A," "C" and "G." The "A" strain is the classical fowl-plague strain used in all the present experiments, the "C" strain is a Newcastle virus, and the "G" strain was possibly also a Newcastle virus. The spleen and liver vaccines included material from each of these three strains in roughly equal proportions. The spleen vaccine consisted of 1 : 30 saline suspensions of the minced organ plus 1 : 2,500 formaldehyde. The liver vaccine was a 1 : 4 suspension of liver tissue in a 60 per cent. solution of glycerine in distilled water, with 0·5 per cent. phenol. Both vaccines had been held at 4° C. for a few weeks (Table I).

Although all three chick-embryo preparations fail to protect in a single dose at eleven days, yet after two doses, both aluminium gel vaccines are effective at 65 days and if anything, the results favour the less alkaline preparation ; in the two-dose group the plain formolised chick-embryo vaccine is still completely ineffective at 65 days.

The result of the immunity test of 19 birds vaccinated with three doses of liver or spleen tissue vaccines is unequivocal. Challenged

at 54 days after the first inoculation (*i.e.*, 40 days from the third injection) two birds only from the spleen-treated group survived and none from the liver-vaccinated group.

TABLE I

COMPARISON BETWEEN FORMOLISED ALLANTOIC FLUID IN BUFFER, Al_2O_3 GELS PREPARED WITH BUFFERS AT *p*H 6·8 AND *p*H 9 AND INACTIVATED TISSUE VACCINE. NUMBER OF BIRDS 36

Type of vaccine	*Dose*	*Interval between vaccination and challenge*	*Number of birds challenged*	*Inoculum*	*Results*	*Number of survivors Number challenged*
		Days				
Allantoic fluid; dilution, 1 : 4 ; buffer, *p*H9 ; formaldehyde, 1 : 2,000	One of 1 c.c.	11	2	A	D4, D4	0/2
	Two of 1 c.c.	16	3	B	D4, two survivors	2/3
		65	6	C	D2, D5, D5, D5, D6, D7	0/6
Allantoic fluid adsorbed on Al_2O_3 gel ; dilution, 1 : 11, *p*H9, formaldehyde 1 : 2000	One of 1 c.c.	11	3	A	D2, D5, D5	0/3
		24	3	D	All survived	3/3
	Two of 1 c.c.	16	3	B	D3, two survivors.	2/3
		65	3	C	D3, two survivors.	2/3
Allantoic fluid adsorbed on Al_2O_3 gel ; dilution, 1 : 11, *p*H6·8, formaldehyde 1:2,000	One of 1 c.c.	11	3	A	D5, D5, 1 survivor	1/3
		24	3	D	D6, two survivors.	2/3
	Two of 1 c.c.	16	3	A	All survived	3/3
		65	3	D	All survived	3/3
Formolised spleen vaccine	Three of 1 c.c.	54	10	C	D2, D2, D3, D4, D4, D5, D5, D5	2/10
Phenolised glycerine, liver vaccine	Three of 1 c.c.	54	9	C	D4, D5, D5, D5, D5, D6, D6, D7.	0/9

D^n signifies one death on the nth day after challenge. This system of notation is followed throughout the tables.

The controls have been collected into one composite Table VII.

Aluminium gel-adsorbed allantoic fluids and embryo tissues

Batch II was used to compare the efficacy of aluminium gel adsorbed vaccines prepared from (*a*) allantoic fluids and (*b*) embryonic membranes and tissues, and to ascertain for how long the immunity evoked by the aluminium gel preparations was likely to persist. One pool of embryos was used for the two vaccines, the tissue-vaccine being prepared by the extraction of the minced membranes and tissues overnight at 4° C. in an equal quantity (w/v) of buffer, followed by centrifuging at 2,000 r.p.m. for 15 minutes. The *p*H

selected was 9·0 since at the time the Batch I tests had not been completed and the more alkaline level was that employed in the preparation of the successful foot-and-mouth disease vaccine (Table II).

To take first the comparison between chick-embryo tissue and allantoic fluid preparations, it is clear that for the period covered

TABLE II

COMPARISON BETWEEN GEL-ADSORBED VACCINES PREPARED FROM ALLANTOIC FLUID AND FROM EXTRACT OF EMBRYONIC TISSUES AND MEMBRANES, EXCLUDING YOLK AND YOLK SAC AND ALBUMEN. NUMBER OF BIRDS 104 AND 65 (*Batches II and III*)

Type of vaccine	*Dose*	*Interval in days*	*Number of birds tested*	*Inoculum*	*Result*	*Number of survivors Number challenged*
Tissue extract. Al_2O_3 gel, 1 : 2, *p*H9, formaldehyde, 1 : 2,000	One of 1 c.c.	9	3	A	All survived (chicks 3 weeks old when vaccinated).	3/3
	Two of 1 c.c.	18	3	D	All survived	3/3
		18	3	D	All survived	3/3
		67	6	C	All survived	6/6
Allantoic fluid; Al_2O_3 gel, 1 : 2, *p*H 9, formaldehyde 1 : 2,000	One of 1 c.c.	9	3	A	All survived (chicks 3 weeks old when vaccinated)	3/3
		18	3	D	D12, 2 survivors	2/3
		18	3	D	All survived	3/3
		54	5	C	D3, D7, 3 survivors	3/5
		188	5	E	D4, two others showed symptoms but recovered	4/5
		222	10	FD	D3, D4, D4, D4, D4, D4, D7	3/10
		222	7	contact	D9, D11	5/7
	Two of 1 c.c.	67	6	C	All survived	6/6
		188	5	E	D6, D6, D7, D8	1/5
		222	13	F	D5, D5, D6, D7	9/13
		222	11	contact	D5, D10	9/11
Allantoic fluid; Al_2O_3 gel, 1 : 2, *p*H9 formaldehyde 1 : 2,000	Two of 1 c.c.	54	5	C	All survived	5/5
		188	5	E	D3, D4 (one other showed symptoms and recovered)	3/5
		222	6	F	D5, D5, D6, D7	2/6
		222	7	contact	D9, D9, D9, D10	3/7
	Two of 1 c.c.	188	5	E	D6, D8	3/5
		222	11	F	D5	10/11
		222	7	contact	D7	6/7

by the immunity tests, the tissue extract (15 survivors out of 15) is at least as effective as allantoic fluids from the same embryos (17 survivors out of 18 birds tested). The comparison does not unfortunately go beyond the 67th day.

On the general question of the duration of the immunity evoked by vaccination with aluminium gel preparations, the tests of Batch

II are quite informative. At 54 to 67 days the immunity conferred by a single dose of 1 c.c. or by two doses of 1 c.c. at seven days interval, remains quite solid (15 survivors out of 17 birds tested), but by the time the next tests are made (188 days) a considerable decline is apparent. Of the single-dose birds tested at 188 and 222 days (roughly six and seven months) there were seven survivors out of 15, while of the birds treated with two doses ten out of 18 survived. Apparently the second dose did not regularly exercise the marked reinforcing effect that is obtained when one repeats the injection of an ordinary inactivated tissue-vaccine, even when the total quantity of vaccine injected is not increased. Possibly the gel adsorbed vaccine, because of its slower rate of adsorption, already provides with the single dose a stimulus so prolonged that the extension induced by repetition of the dose seven days later is not always of great significance.

It will be observed that at 222 days seven birds of the single-dose group and eleven of the two-dose group were not inoculated with a test dose of virus but were exposed to contact infection. The survival rate (five out of seven, and nine out of eleven respectively was roughly 50 per cent. higher than that of the corresponding groups tested by inoculation of virus. The contact exposure was severe since 96 fowls, including birds vaccinated with Batches II, III, V and VII and susceptible controls, were mixed in two loose boxes 60 of them being inoculated with virus. Twenty-six deaths occurred among the inoculated birds three to nine days later. Among the 32 vaccinated birds exposed by contact the first death occurred on the fifth day, the second on the seventh day, four on the ninth day, two on the tenth day and one on the eleventh day. From the eleventh day onwards there were no further cases and it is assumed that the infection died out. It seems certain that, as a test of immunity, contact exposure is less severe than the method of inoculation and that partially immune birds that succumb late to injection of virus might possess sufficient immunity to resist this method of infection.

From the tests made with Batch II it may be concluded that an extract of virulent embryonic tissues and membranes is probably as effective an immunising agent as virulent allantoic fluid, and that the immunity evoked by one or two doses of gel-adsorbed vaccine has begun noticeably to decline by six months.

Aluminium gel-adsorbed allantoic fluid pH 9·0

Batch III, a gel-adsorbed vaccine prepared from a pool of allantoic fluid at *p*H 9·0, was similarly utilised to test the duration of immunity. The results at 188 and 222 days are not very different from those of Batch II, except that the two-dose group regularly shows a marked superiority to the single-dose group when exposed either by contact or inoculation at 222 days (Table II).

The results of a few tests with Batches V, VI and VII, are included, since they were made at the critical period when the immunity from the gel vaccines begins to decline. Batch V, allantoic fluid gel, shows a declining immunity at 172 days; Batch VI prepared from embryos, membranes and fluids appears to be more potent, while Batch VII which included yolk and yolk-sac seems of about the usual level. The lower virus-content of yolk has been evident in direct titrations that have been made (Table III).

TABLE III

TESTS MADE WITH GEL-ADSORBED VACCINES WHEN IMMUNITY IS DECLINING

Type of vaccine	*Dose*	*Interval of days*	*Number of birds tested*	*Inoculum*	*Result*	*Number of survivors / Number challenged*
			Batch V			
Allantoic fluid I : II, One of Al_2O_3 gel, *p*H 8·0, formaldehyde 1 : 2,000	1 c.c.	172	5	F	D3, D6, D6	2/5
			Batch VI			
Embryos membranes, allantoic fluid, I : II, Al_2O_3 gel *p*H 6·8, formalised 1 : 2,000 (excluding albumen and yolk)	One of 1 c.c.	172	3	F	All survived	3/3
			Batch VII			
Embryos, fluids, membranes, including yolk and yolk sac, but excluding albumen; 1 : 11, *p*H 6·8, Al_2O_3 formaldehyde 1 : 2,000	One of 1 c.c.	172	2	F	D4	1/2

Oily base vaccines

Batch IV, another allantoic fluid pool, was used to prepare a vaccine with liquid paraffin, lanolin, beeswax and killed acid-fast bacteria. The tubercle bacilli were of a virulent human strain and were dried on the Cryochem desiccator after being heated in the autoclave for one hour at 120° C. The proportions of lanolin, beeswax and mineral oil were those shown in Table IV. The weighed amount of dried bacteria was rubbed into suspension in a small quantity of the liquid paraffin and then mixed with the remainder of the paraffin. The lanolin and beeswax were melted and mixed

and added to the paraffin which had previously been heated to 50° C. Finally the suspension of virus was added to the mixture at a temperature not exceeding 40° C., and the whole was emulsified with a turbo-agitator into a creamy water-in-oil emulsion. The virus-bearing tissues and fluids are contained in the dispersed watery phase, while the acid-fast organisms are suspended in the continuous oily phase, as may be readily verified by microscopic examination of suitably stained samples.

TABLE IV

COMPARISON BETWEEN OILY-BASE VACCINES WITH ACID-FAST BACILLI, Al_2O_3 GEL-ADSORBATE VACCINE, AND PLAIN FORMOLISED ALLANTOIC FLUID, C.E.* TITRE, ALLANTOIC FLUID $10^{9 \cdot 5}$. NUMBER OF BIRDS 120

Batch IV

Type of vaccine	*Dose*	*Interval in days*	*Number of birds tested*	*Inoculum*	*Result*	*Number survived Number challenged*
Allantoic fluid formolised 40 per cent.; mineral oil containing dried tubercle bacilli 4 mg. to 1 c.c., 40 per cent.; lanolin 15 per cent.; beeswax 5 per cent.	One of 1 c.c.	24	4	C	—	4/4
		159	4	E	—	4/4
		193	3	F	D6	2/3
		305	2	G	—	2/2
		427	2	I	D4	1/2
	One of 0·5 c.c.	24	5	C	—	5/5
		159	2	E	—	2/2
		193	3	F	—	3/3
		305	3	G	—	3/3
		427	2	I	—	2/2
Allantoic fluid, formolised 40 per cent.; aluminium gel, 33 per cent.; phosphate buffer, *p*H 6·8 27 per cent.	One of 1 c.c.	24	5	C	—	5/5
		159	5	E	D5, D7, D8	2/5
	One of 0·5 c.c.	24	5	C	—	5/5
		159	5	E	D8	4/5
Allantoic fluid, formolised 40 per cent.	One of 1 c.c.	24	5	C	D4, D7	3/5
		159	5	E	D3, D4, D4, D5, one each recovered	1/5
Phosphate buffer, *p*H 8 60 per cent.	One of 0·5 c.c.	24	5	C	D3, D5, D8, D9	1/5

* C.E. = chick red cell agglutination.

From the same pool of allantoic fluid an aluminium gel adsorbate, and a plain formolised vaccine in buffer were also prepared for comparative tests. It will be noticed that the virus concentration is equal in all three vaccines and that it is roughly five times as great, in terms of infective allantoic fluid, as that of the gel vaccines Batches I, II and III; so that a dose of 0·5 c.c. contains about two and a half times as much virus as 1 c.c. of Batches II or III.

Fifteen days after vaccination, sera from birds that had received the oily vaccine inhibited haemagglutination to a titre of 1 : 320, the first time such inhibition had been demonstrated so soon after vaccination, while sera from the group inoculated with the aluminium gel and plain formolised vaccines did not inhibit at 1 : 80 the lowest dilution tested. At 24 days, 9 birds in the oily-vaccine group and ten in the aluminium gel group completely resisted a challenge dose of virus ; whereas out of ten birds that had received the plain formolised vaccine six died after the challenge inoculation. At 159 days, when a further small sample was drawn for test, six of the oily vaccine group survived without symptoms, but of ten birds of the aluminium gel group, four died, and four out of five birds died in the test of the plain formolised group. The behaviour of the birds vaccinated with the aluminium gel preparation is comparable with that of birds vaccinated with single doses of Batches II and III and it is clear that here again by the sixth month the immunity has declined considerably from the high level attained during the first two months.

In later tests of the oily vaccine group one bird out of five tested 193 days after vaccination died on the sixth day, and five birds tested at 305 days all resisted the challenge. Finally, four birds of this group were challenged after 427 days ; three survived, two of them from the 0·5 c.c. group and one from the 1 c.c. group. The total number challenged in this group was 30 and the survivors 28.

In the meantime, a number of other tests have been made of the oily base vaccine, using Batch IV and other batches. In one small experiment, birds vaccinated with Batch IV were challenged on the first to the eighth days after vaccination, one on each day. The first seven died, but the bird challenged on the eighth day resisted completely. Another group of seven birds treated with Batch IV were challenged, one on the eighth day, one on the ninth, one on the tenth and four on the 40th days ; all completely resisted the challenge. It appears that sufficient immunity to withstand a challenge by inoculated virus is not developed until the eighth day, but from then onwards the immunity is solid.

Oily vaccines with different antigens

The next group of trials, employing dilutions, was designed primarily to compare the value of infective allantoic fluids, embryonic tissues and membranes and yolk and yolk-sac as sources of antigen for the oily vaccine, and to obtain indications regarding dosage. The opportunity was also taken to compare the efficacy of vaccines with and without acid-fast bacilli. The vaccine, Batch XXVII, was prepared from a pool of embryos in which the titre of the allantoic fluid was $1 \times 10^{-7.5}$, diluted in phosphate buffer at *p*H 6·8. Each c.c. of vaccine contained 0·4 c.c. virus material diluted or undiluted, 0·4 c.c. of liquid paraffin containing 4 mg. of

dried tubercle bacilli per c.c. and 0·2 c.c. of beeswax and lanolin 1 : 7. The dose was 0·5 c.c. intramuscularly. The results are tabulated in Table V. Half the birds were challenged with A strain-T/37 allantoic fluid 1 c.c. × 10^{-6} 31 days, and half with A strain-T/39 allantoic fluid 1 c.c. × 10^{-6}; CE titre $10^{-9.5}$ 121 days after vaccination. There were no deaths among the four groups, 40 birds in all, vaccinated with the undiluted materials containing acid-fast bacteria. In the 1 : 5 dilutions (1 : 10 in the case of the tissue vaccine) there were no deaths in the allantoic fluid and tissue groups and only one death out of nine birds in the yolk and yolk-sac group. In the higher dilutions there were two deaths out of nine birds in the allantoic fluid group, and three birds out of eight in the yolk and yolk-sac group, while all ten birds in the 1 : 50 embryonic tissue group resisted the challenge. These results fully bear out the indications of the earlier tests that embryonic tissues and membranes are superior to allantoic fluid as a source of antigen and that yolk and yolk-sac are the least efficacious of the egg components. They also suggest that a vaccine prepared from whole embryos (less albumen) might prove adequate as an immunising agent, when diluted with an equal part of buffer or saline.

TABLE V

COMPARISON (A) BETWEEN ALLANTOIC FLUID, EMBRYONIC TISSUES AND MEMBRANES, AND YOLK AND YOLK SAC, AS SOURCES OF VIRUS FOR OILY-BASED VACCINE; AND (B) BETWEEN OILY-BASED VACCINES PREPARED FROM THE COMMON POOL OF ALLANTOIC FLUIDS WITH AND WITHOUT KILLED MAMMALIAN TUBERCLE BACILLI

Batch XXVII

	Tested at 31 *days*		*Tested at* 121 *days*		*Survivors challenged*
	Number	*Result*	*Number*	*Result*	
A : *Preparation, including killed mammalian tubercle bacilli* :					
Allantoic fluid :					
Undiluted	5	Nil	5	Nil	10/10
Diluted 1/5	5	„	5	„	10/10
„ 1/25	5	D3, D7	4	„	7/9
Tissues and membranes :					
Diluted 1/2	5	Nil	5	Nil	10/10
„ 1/10	5	„	4	„	9/9
„ 1/50	5	„	5	„	10/10
Yolk and yolk sac :					
Undiluted	5	Nil	5	Nil	10/10
Diluted 1/5	5	„	4	D3	8/9
„ 1/25	5	D7	3	D3, D4	5/8
B : *Allantoic fluid without killed tubercle bacilli* :					
Undiluted	5	Nil	5	Nil	10/10
Diluted 1/5	5	D3, D4	3	D4, D6	4/8
Controls	4 +	D3, D3, D3, pens group G : 16 birds			1/20

+ : Birds bought on open market, one apparently immune to fowl plague.

The omission of the acid-fast bacilli plainly reduced the efficacy of the allantoic fluid preparation, since four out of eight birds in the 1 : 5 group died when the vaccine did not contain acid-fast bacilli, whereas when the latter were included all birds in the 1 : 5 group and seven out of nine in the 1 : 25 group survived.

On the basis of these results a vaccine prepared from the whole contents of the egg (less albumen), diluted with an equal quantity of buffer or normal saline, was adopted for routine use. A further modification has been the substitution of a much lighter mineral oil for the liquid paraffin originally employed. The latter has a viscosity of 427 seconds at 70° F. Redwood, and was too heavy for convenient use in the field in the winter months. It has been replaced by P 40, a white oil with a viscosity of 120 at 70° F. Redwood (supplied by the Shell company). With this modification the vaccine is easy to inject if maintained at a temperature of about 30° C. The yield of vaccine by this method of preparation is approximately 200 doses of 0·5 c.c. from an average Egyptian egg weighing approximately 40 to 42 g. before incubation. The records of two batches prepared by this technique will serve as examples.

Group A constitutes a small random sample of six birds inoculated with Batch XXIX (undiluted), drawn from a farm inoculation of 2,000 young susceptible birds: all these birds were resistant. Group B was inoculated with Batch XXX (B). It will be observed that the earlier promise is borne out by the resistance of all birds in groups inoculated with 0·5 c.c., including those receiving dilutions of 1 : 25. Since the original virus material was already diluted with an equal part of saline, the strengths of virus material actually injected were 1 : 2, 1 : 10 and 1 : 50 respectively (Table VI).

At the start of this particular experiment 100 young birds susceptible to fowl plague and Newcastle disease were introduced from a healthy flock. The object was to demonstrate by later challenge with Newcastle disease virus, that birds protected against fowl plague still remained fully susceptible to Newcastle disease ; but that when the two vaccines were administered simultaneously the birds would acquire immunity against both diseases. Forty of these birds were spread over eight groups, five in each, the groups being dilutions 1/5 and 1/25 of Batch XXX (B) all three groups of XXX (C) and all of XXX (D). Twenty more of these young birds were inoculated with XXX (B), and were given a dose of Newcastle vaccine simultaneously (Table VI).

A chance introduction of Newcastle disease to the premises resulted in the loss of 73 birds in a matter of 15 days. The 20 birds vaccinated simultaneously against fowl plague and Newcastle disease all survived the outbreak, as did all the remaining birds in the pens. There were approximately 500 birds in this group all of which had been immunised with Newcastle disease vaccine about ten months previously, and they included the flock of roughly 400

laying hens, susceptible to fowl plague, that provides eggs for the virus work at the Institute. Since the 20 birds that had received the two vaccines simultaneously survived the natural infection and later resisted the challenge with fowl-plague virus, they will serve as an indication of the efficacy of simultaneous vaccination which is now frequently practised in the field in Egypt.

TABLE VI

TRIALS OF VACCINES PREPARED FROM WHOLE EGG CONTENTS (LESS ALBUMEN), DILUTED WITH AN EQUAL QUANTITY OF UNBUFFERED NORMAL SALINE SOLUTION
Batches XXIX and XXX

Vaccine batch	*Dose*	*Interval*	*Number challenged*	*Challenge*	*Result*	*Survivors challenged*
		Days	Random of sample			
XXIX. Field inoculation of 2,000 birds	0·5 c.c.	22	6	1 c.c. × 10^{-6} A52 CE titre 10^{10}	—	6/6
Controls for AXXIX Fayoumi	—	—	3	„	D3; D3, D4	0/3
XXX (B) Undiluted	0·5 c.c.	26	16	G	—	16/16
Diluted 1 in 5	0·5 c.c.	30	5	H	—	5/5
Diluted 1 in 25	0·5 c.c.	30	5	H	—	5/5
Undiluted (simultaneously with Newcastle vaccine)	1 c.c.	23	20	G	—	20/20
XXX (C) *Prepared from the same pool, but with an equivalent weight of dried killed* Mycobacterium butyricum *in place of the mammalian tubercle bacilli incorporated in B XXX*						
Undiluted	0·5 c.c.	30	5	H	D7	4/5
Diluted 1 in 5	0·5 c.c.	30	5	H	D2, D3	3/5
Diluted 1 in 25	0·5 c.c.	30	4	H	D3, D3, D6, D7	0/4
XXX (D) *Prepared from the same embryo pool, incorporating killed mammalian tubercle bacilli, but with the substitution of additional P40 oil lanolin and beeswax in B XXX*						
Undiluted	0·5 c.c.	30	5	H	—	5/5
Diluted 1 in 5	0·5 c.c.	30	5	H	—	5/5
Diluted 1 in 25	0·5 c.c.	30	5	H	D3, D5	3/5

The table also includes the record of trials of a vaccine prepared from the same pool of infective materials, but with a strain of *Mycobacterium butyricum* from the National Collection of Type Cultures, Elstree, in place of the tubercle bacilli normally employed, and of vaccines from which the lanolin and beeswax have been omitted.

The substitution of dried killed *M. butyricum* of this particular strain for the mammalian tubercle bacilli appears to reduce the

efficacy of the vaccine to about the same extent as did the complete omission of the acid-fast organisms from Batch XXVII. Apparently this does not apply to all strains of *M. butyricum,* since Friedewald (1944) obtained even higher titres in mice with an influenza vaccine incorporating *M. butyricum* than with vaccines containing tubercle bacilli.

When the lanolin and beeswax XXX (D) are omitted and their place is taken by additional P40 oil, the emulsion is no longer completely stable and there is a sensible reduction in the potency of the preparation, as witnessed by the behaviour of birds inoculated with the 1 : 25 dilution.

DISCUSSION

It is remarkable that by emulsification with a mineral oil, lanolin and beeswax, and the addition of killed acid-fast bacilli, one can transform an antigen, as ineffective by itself as formolised infective allantoic fluid proved to be in the earlier experiments, into a vaccine of which a single small dose is capable of evoking a solid immunity that persists for as long as 10 to 14 months. A similar transformation was obtained by Friedewald (1944) with infective allantoic fluids or watery suspensions of purified influenza virus. Brandly *et al.* (1946) also recorded an augmentation and prolongation of the immunity response of chickens to vaccination with inactivated virus of Newcastle disease when similar adjuvants were added, but concluded " With those agents not producing an unduly severe and protracted tissue reaction, the degree or duration of immunity was, however, not regularly enhanced to a satisfactory degree."

No doubt the pronounced adjuvant effect of the mineral oil-lanolin combination and the prolongation of the period of immunity is largely bound up with the slow rate of adsorption of the materials and the local stimulation of a monocytic reaction. But in what manner the addition of the killed acid-fast bacilli enhances the immunogenic properties of antigens so diverse as dysentery bacilli and filtrable viruses is not yet clear.

Halbert *et al.* (1946) showed that an antigen (*S. dysenteriae*) in water-in-oil emulsion, injected subcutaneously into mice, persisted at the site of inoculation for a period of 24 weeks ; and more recently Herdigen *et al.* (1947) succeeded in recovering and titrating small amounts of such antigen from the persisting depot at 18 to 24 weeks, the amount recovered depending upon the quantity of antigen originally injected.

In the present experiments, the depots of oily vaccine have been found to persist exceptionally for as long as 30 weeks. Only a few of the original birds have come to *post-mortem* examination, but in general it may be stated that a few days after inoculation, the oily vaccine injected intramuscularly in the pectoral muscles

tends to be deposited in a longitudinal pocket formed between the muscles or fibre groups and lying in contact with the fascia. There is no capsule and no sign of inflammatory reaction except possibly a slight subcutaneous oedema overlying the area of the deposit. By the sixth week the material is completely enclosed within a thin elongated capsule, and has undergone considerable inspissation, being crumbly in consistency. There is still no sign of inflammatory reaction in the muscular tissues surrounding the capsule. At this stage the spindle-shaped capsule may be about 2 in. long and up to ⅓ in. in greatest diameter. The depot found after 25 weeks was about an inch long, flattened and up to ¼ in. at the maximum in thickness, the contents being quite dry and crumbly. The fate of oily vaccine injected subcutaneously is much the same. For the first few days the mineral remains spread diffusely in the subcutaneous tissue and there is some evidence of local oedema ; but by the sixth week the subcutaneous depot has been aggregated to form an encapsulated, nodular mass which may approach the size of a walnut. The capsule is thicker than that of the intramuscular depot, but the contents are subject to the same progressive process of inspissation. The degree of local reaction is astonishingly slight in both cases.

As to the persistence of the depot, out of seven birds inoculated intramuscularly from 25 to 30 weeks previously, residual vaccine was found in three only, one at 25 weeks, one at 19 weeks and one containing very little material at 30 weeks. Three birds at 25 weeks and one at 29 weeks showed no residue. Attempts to demonstrate the persistence of antigen in the residuum by vaccinating susceptible birds with the re-suspended material have so far failed, in one case even when the residual material was only seven weeks old. The result is hardly surprising if one accords due weight to the difficulty with which susceptible birds are immunised against this virus.

The inoculation of healthy birds with material containing killed mammalian tubercle bacilli does not produce any ill effect, neither does a second inoculation with the same material whether given a few days, a few weeks or several months after the first injection. The effect on birds already infected with tuberculosis has not been tested.

Since injection into the pectoral muscles undoubtedly spoils the carcase for several months, as a routine method the vaccine has been given subcutaneously on the inside of the thigh or at the base of the neck dorsally. From these sites the vaccine depot can readily be removed without damage to the carcase.

Upwards of 150,000 birds have already been inoculated with the oily-base vaccine containing mammalian tubercle bacilli without ill effects and a large proportion have been given Newcastle disease

vaccine simultaneously. So far there have been no cases of natural infection in vaccinated birds, although in recent months natural outbreaks of fowl plague have been encountered in two provinces. Two outbreaks of fowl plague in large flocks have been successfully suppressed by vaccination coupled with simple measures of segregation.

TABLE VII

CONTROL BIRDS

Inoculum	*Description*	*Number of birds*	*Result*
A	Allantoic fluid, 42nd passage "A" 1 c.c. × 10	2	D3, D5
B	Allantoic fluid, 46th passage "A," 1 c.c. × 10^{-6}	3	D3, D3, D3
C	Allantoic fluid "A" (T. 10) 1 c.c. × 10^{-6}	10	D3, D4, D4, D4, D4, D3, D4, D5, D6, D8
D	Unformolised gel, +CE titre $10^{-5.5}$ dose 1 c.c. undiluted	1	D4
E	Allantoic "A" (T24) 1 c.c. × 10^{-6}	10	D2, D3, D3, D3, D3, D3, D3, D4, D4, D4
F	Allantoic fluid "A" (T29) 1 c.c. × 10^{-6}	4	D3, D3, D3, D4
G	Allantoic fluid "A" (T39) 1 c.c. × 10^{-6}	16	D2, D2, D3, D3, D3, D3, D3, D3, D3, D3, D3, D3, D4, D4, D5.
H	Allantoic fluid "A" (T43) 1 c.c. × 10^{-6}	7	D3, D3, D3, D3, D4, D5.
I	Allantoic fluid "A" (T51) 1 c.c. × 10^{-6}	3	D3, D3, D3.

+ This gel had been stored for 18 days at 4° C. when used in the immunity tests. At 20 days, *i.e.*, two days later, it was roughly titrated on young chicks and killed one out of two chicks in 24 hours at a dose of 1 c.c. × $10^{-5.5}$, four chicks inoculated with lower dilutions all died within 48 hours.

CONCLUSIONS

The following conclusions may be drawn from the work at its present stage.

Inactivated tissue-vaccines prepared from the tissues of infected birds by the methods of Staub (1926) and Todd (1928) do not constitute a reliable immunising agent against fowl plague.

Plain formolised vaccines prepared from the allantoic fluids of chick embryos in which the virus has been cultivated, are more antigenic than vaccines prepared from the tissues of infected birds, but the protection they afford is transient and birds that have been vaccinated twice may become fully susceptible within 65 days.

Formolised vaccines prepared from tissues and fluids of chick embryos in which the virus has been cultivated, can confidently

be used for the immunisation of chicks and fowls from the age of three weeks upwards, provided certain adjuvant substances are incorporated in suitable quantity in the vaccine.

When Willstätter's aluminium hydroxide gel is employed as an adjuvant in the proportion of 1 : 2 (33 per cent. of the whole vaccine), with virus-containing tissues or fluids at a final strength of 1 : 11, the buffered vaccine so prepared will immunise birds with considerable regularity.

Birds injected with 1 c.c. of the aluminium gel vaccines are usually immune from two weeks onwards, and the immunity is solid at two months. Six months after vaccination immunity is definitely on the decline. The immunity evoked by a single dose of aluminium gel vaccine is less uniform than that evoked by two doses at one week's interval and it disappears more rapidly. In the tests outlined in this report 75 birds out of 85 resisted a challenge injection of virus eleven days after treatment with either one or two doses of aluminium gel vaccines, and 47 out of 75 birds resisted a similar challenge 159 to 222 days after vaccination. When exposure was by contact 23 birds out of 32 resisted at 222 days.

Vaccines prepared in the form of a water-in-oil emulsion, consisting of formolised chick-embryo material, mineral oil, lanolin, beeswax and killed mammalian tubercle bacilli, evoke a solid immunity in birds eight days after the injection of a single dose of 0·5 c.c. In a comparative test a vaccine so prepared proved definitely superior to an aluminium gel vaccine made from the same pool of infected allantoic fluids. Of 38 birds so far challenged with virus between the eighth and 427th days after vaccination with this particular batch, only two have died, one at 193 days and one at 427 days.

Of the egg components, embryonic tissues and membranes furnish the most potent antigen, allantoic fluids the next and yolk and yolk-sac the least potent ; but a satisfactory vaccine can be prepared from whole egg-contents (less albumen) diluted with an equal quantity of saline or buffer. A vaccine so prepared will protect birds in a dose of 0·5 c.c. (52 survivors out of 52 birds tested in this group), even when the virus component has been diluted 1 : 25.

By the omission of the killed acid-fast bacilli or by the substitution of one particular strain of *M. butyricum* for the mammalian tubercle bacilli, the efficacy of the vaccine is considerably reduced. The substitution of additional mineral oil for the lanolin-beeswax component also reduces antigenic efficacy, but not to the same extent as the omission of the acid-fast bacilli.

In birds treated with the oily-base vaccine a depot of material persists for some months at the site of inoculation. So far it has not been possible to demonstrate the persistence of antigen in the residual material.

Apparently the domestic fowl is not readily sensitised by the injection of killed mammalian tubercle bacilli in the quantities employed in these tests and the vaccination may be repeated after a short or longer interval without ill effect.

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Mr. R. Daubney has drawn the attention of the Editors to the fact that this paper together with one on "The Occurrence of Newcastle Disease in Egypt" which appeared in the issue of July, 1948, have been published in the Bulletin d'Office Internationale des Epizooties although Professor Léclainche, the Director of the Bureau, had been informed by Mr. Daubney that they had been offered to the JOURNAL OF COMPARATIVE PATHOLOGY. The papers were accepted by the Editors on the assurance that they would not be published elsewhere. The authors have intimated that they were under a complete misapprehension when this assurance was given. They desire to offer their unreserved apologies to all readers of the Journal for this occurrence. The Editors would add their regrets that Mr. Daubney's intimation was received too late to enable them to withhold publication.

EXPERIMENTS ON THE TRANSMISSION OF *STREPTOCOCCUS AGALACTIAE* INFECTION BY MILKING WITH INFECTED HANDS

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INTRODUCTION

The transmission of bovine mastitis due to *Str. agalactiae*, Group B (Lancefield, 1933) and the epidemiology of the disease have received the attention of a number of workers. It is probable that one of the greatest handicaps encountered has been the difficulty of experimentally reproducing the varied conditions found in dairy herds. It is not claimed that in the experiments to be described all these difficulties were overcome, but within the limits of the experiment, conditions closely resembling those occurring in the field were maintained for a period of four months. Milking was by hand and at no time was any artificial treatment of the udder or teats introduced as, for example, experimental damage to the orifice or incomplete milking.

It is now generally accepted that pathogenic streptococci gain access to the udder by means of the teat canal, but the relative importance of the factors influencing infection is not so well established. Workers studying this question have adopted three main methods of approach, *viz.*, injection of infective material *via* the teat canal ; application of infective material to the teat, sometimes enclosed in bags, on one or more occasions ; and milking with infected hands. Only the last two methods have a direct bearing on our experiment and the survey of the literature will be confined to these aspects and to those in which the teat was not damaged.

Although a number of authors have failed to set up infection (Rienhoff, 1929 : Seelemann, 1929, 1932a and b : Seelemann and Siemonsen, 1932a ; Schuman and Lorenzen, 1933 ; Hopkirk, 1935 : Bendixen, 1934 ; Rosell, 1931 ; Little, 1937 ; Starr *et al.*, 1936 : Christiansen and Nielsen, 1934), occasionally it has been produced (Seelemann and Siemonsen, 1932b ; Udall, 1936 ; Klimmer and Haupt, 1933 ; Peterson and Hastings, 1939 ; Bryan, 1938 ; Little, 1937). McEwen and Samuel (1946) studied the artificial infection of the teat canal with a strain of *B. coli*. Several authors (Seelemann, 1932b ; Stableforth *et al.*, 1935 ; Murphy, 1947, and others) have shown that the incidence of *Str. agalactiae* infection rises with age.

The genesis of these experiments was a wish to compare the susceptibility of animals to infection and to use as natural a method as possible, that is one other than injection beyond the teat barrier.

It will have been gathered, however, from the literature surveyed, that previous attempts to transmit *Str. agalactiae* in this way have had varied success. It was decided, therefore, to make preliminary experiments to discover what degree of infection could be expected from a heavy and prolonged exposure to milking with hands infected with secretion.

It is well known from herd observations that some animals appear to be more susceptible than others and it seemed possible that one reason for the varied success referred to above might have been differences in the susceptibility of the animals used. When heifers were employed no guidance from their past history would be available, whereas cows might be selected because of their past freedom from infection which could be due to an inherent resistance. It was decided, therefore, to include a group of animals which had previously been infected and cured, in addition to previously clean heifers. It was intended that these previously infected animals should also be young stock but, at the time they were needed it was found impracticable to obtain young animals previously infected with a serological type of *Str. agalactiae* distinct from that which it had been decided to use for infection, and a group of older cows had therefore to be used. In addition to the two groups already mentioned, a group of second calvers which been used for other experiments in the previous year were also included.

The bearing of the differences in age on the results will be discussed later.

DETAILS AND MANAGEMENT OF ANIMALS USED IN THE EXPERIMENT AND SAMPLING METHODS

Animals used

Group I (*First calving heifers*). This group consisted of four Ayrshires (963, 965, 968, 971) and three Friesians (966, 967, 969). There was no history of previous mastitis nor of any streptococcal infection from the time of calving to the commencement of the experiment. Bacteriological examination of milk samples showed haemolytic staphylococci to be present in two quarters of one heifer. All these seven heifers had calved within the eight weeks prior to the commencement of the experiment.

Group II (*Second calvers*). This consisted of five second calf Ayrshires (756, 758, 759, 766, 767). During the first lactation these cows had shown no previous mastitis and streptococci and haemolytic staphylococci were absent from milk samples taken during this period (for bacteriological technique, *vide infra*). In the case of four cows the maximum interval between calving and the beginning of the experiment was two months ; the fifth cow calved three weeks after the commencement. During the interval between the second calving and commencement of the experiment, frequent

bacteriological examination of the milk samples from the four cows showed three quarters were harbouring haemolytic staphylococci and one quarter was infected with *Str. uberis.*

Group III (*Older cows*). This group consisted of three Shorthorns (928, 929, 930) and three Friesians (962, 964, 970) in their fourth to seventh lactation. Five of these cows had been previously infected with *Str. agalactiae* type Ib (Stableforth,1932, 1937). During the preceding lactation this infection had been removed by intramammary infusion, as proved by repeated bacteriological examinations. The sixth cow (964), in her sixth lactation, had shown no established *Str. agalactiae* infection during her previous two lactations; bacteriological data for her first three lactations were not available. Before the commencement of the experiment, small numbers of haemolytic staphylococci were detected in the milk from one quarter of these six cows but no streptococci were found. In four cows, the maximum interval between calving and the beginning of the experiment was three months: the remaining two calved within five weeks after the experiment had commenced.

Donor cows (895 *and* 928). Cow (895) was an Ayrshire in her fourth lactation, calving in the second week of the experiment, that is, the week when exposure began. Four tests made during the previous lactation showed her to be heavily infected in all four quarters with *Str. agalactiae,* type IIIa. This strain was of distinct colonial appearance and the infection remained in all quarters during the time she was used. Haemolytic staphylococci were absent from all samples. This cow was used until 26.9.46 (*i.e.,* during the eleventh week of the experiment) and was replaced thereafter by a new donor (928), one of the cows which became infected during the experiment.

Management

The 18 animals in Groups I, II. and III (this excludes the original donor cow 895) were permanently housed in a modern type cow byre. They were bedded in deep straw and received a good production ration of hay, roots and concentrates. Once daily, after the morning milking, the udder and hind quarters of each were thoroughly washed with cold water from a hose-pipe and then left to dry. Before each milking the udders were washed with a cloth and warm water. No disinfectant of any kind was used in the byre. Separate attendants were available and before the commencement of the experiment *Str. agalactiae* was absent from hand washings of each milker.

The procedure was as follows. At each milking the clotted secretion from the RH (right hand) quarter of the donor cow (895) was taken into an enamel container. Milkers dipped their hands into this container, applied the secretion remaining on their hands

to the teats and lower parts of the udder and immediately proceeded to milk. Three milkers were employed and milking was so arranged that each cow was milked approximately an equal number of times by each individual. For the sake of brevity this procedure will be subsequently referred to as milking with infected hands.

After milking the weight of bulk milk was recorded. Milking was commenced at a different end of the cow byre on alternate weeks, at 8 a.m. and 3.30 p.m. daily.

The experiment commenced on 27.6.46. Fourteen days later, at the afternoon milking, the cows were first exposed to *Str. agalactiae*. The exposure lasted 15 weeks 4 days. For 14 days prior to commencement of exposure, ten daily quarter samples and during the experiment 68 quarter samples were taken from each lactating cow. The experiment terminated on 28.10.46, the cows being then required for further experiments of a different nature.

Collection of Samples

Quarter samples were taken before the morning milking on four or five days each week. Immediately before the sampling, the teats were swabbed with sterile cotton wool moistened in sterile water. The first draw of the fore milk was examined for clots and discarded. For the bacteriological examination, about 15 c.c. of milk was taken into a sterile 1 oz. McCartney bottle.

Laboratory Methods

Bacteriological examination of milk samples was made within a few hours of collection. One-tenth of a c.c. of whole milk from each sample was spread on to the surface of sheep blood agar plates. These were incubated for 48 hours at 37° C., the colonies identified by their macroscopical appearance and the number estimated. As each quarter became infected the serological type of the *Str. agalactiae* isolated was determined. The remainder of the milk sample was incubated for 18 hours at 37° C. One loopful was then streaked on to the surface of one half of a plate containing crystal violet, aesculin blood agar (Edwards, 1933), and the results recorded after 24 hours at 37° C. Whiteside and electro-conductivity readings were also made daily.

RESULTS

Organisms recovered

Str. agalactiae. The number and percentages of cows and quarters found infected with *Str. agalactiae* for the various weeks after exposure to infection are shown in Table I. The bacteriological findings are based on the results of the direct plate examination of

0·1 c.c. of the milk sample and do not include the results following enrichment, which will be dealt with later. As stated above, for two weeks the cows were not exposed to *Str. agalactiae* infection and samples taken during this period showed *Str. agalactiae* to be absent from all quarters.

It will be seen that during the first week after commencement of exposure two quarters of two older cows (964, 929) in Group III became infected with *Str. agalactiae*. At the fifth week the number of older cows which had become infected had risen to five (928, 929, 930, 962, 964) and they were infected in a total of eight quarters. One quarter of one second calver (758) also became infected. By the tenth week the remaining older cow (970) in Group III was infected. There was no increase in the number of infected cows in Groups I and II. By the 13th week, five quarters of three second calvers (758, 766, 767) and three quarters of two heifers (966, 968) had become infected. At this time there was also an increase from 13 to 17 in the number of infected quarters in the older cows. One week later the fourth cow (756) in Group II became infected in one quarter, and in the final week a further quarter of this cow became infected.

TABLE I

Str. agalactiae INFECTIONS PRESENT IN SUCCESSIVE WEEKS AFTER EXPOSURE TO INFECTION IN COWS MILKED WITH INFECTED HANDS

Weeks after exposure to infection	*Group I* 7 *heifers*			*Group II* 5 *second calvers*			*Group III* 6 *older cows*			*Totals* 18 *cows* 69 *quarters*		
	Total cows infected	*Total qtrs. infected*	*% qtrs.*	*Total cows infected*	*Total qtrs. infected*	*% qtrs.*	*Total cows infected*	*Total qtrs. infected*	*% qtrs.*	*Cows infected*	*Qtrs. infected*	*% qtrs. infected*
1	0			0			2	2	10	2	2	3
2	0			0			4	5	24	4	5	7
3	0			0			4	5	24	4	5	7
4	0			0			4	6	29	4	6	9
5	0			1	1	5	5	8	38	6	9	13
6	0			1	1	5	5	9	43	6	10	14
7	0			1	1	5	5	12	57	6	13	19
8	0			1	1	5	5	12	57	6	13	19
9	0			1	1	5	5	12	57	6	13	19
10	0			1	2	10	6	13	62	7	15	22
11	0			1	3	15	6	13	62	7	16	23
12	0			1	3	15	6	13	62	7	16	23
13	2	3	11	3	5	25	6	17	81	11	25	36
14	2	3	11	4	6	30	6	17	81	12	26	38
15	2	3	11	4	7	35	6	17	81	12	27	39

Thus, at the termination of the experiment, 27 quarters of twelve cows had become infected with *Str. agalactiae* and these comprised four out of five second calvers (Group II), six out of the six older cows (Group III) and two out of the seven heifers (Group I).

Table II shows the interval between the dates of calving and the commencement of exposure to infection for each cow together with the number of cows to become infected. This interval did not seem to have any bearing on the relative susceptibility of the cows.

TABLE II

THE RELATION OF DATE OF CALVING TO SUBSEQUENT INFECTION

Interval between calving and commencement of exposure to infection	*Number of cows*	*Number of cows to become infected*
2–4 days	3 (Nos. 758, 928, 929)	3
1 week	—	—
2 weeks	1 (No. 767)	1
3 „	2 (Nos. 967, 970)	1 (No. 970)
4 „	—	—
5 „	—	—
6 „	1 (No. 966)	1
7 „	3 (Nos. 968, 969, 971)	1 (No. 968)
8 „	—	—
9 „	1 (No. 964)	1
10 „	4 (Nos. 759, 766, 963, 965)	1 (No. 766)
11 „	2 (Nos. 756, 962)	2
12 „	—	—
13 „	—	—
14 „	—	—
15 „	1 (No. 930)	1

Haemolytic staphylococci. As indicated in an earlier part of this paper, haemolytic staphylococci were present in a total of six quarters of five cows before the experiment began: five quarters showed this organism for the duration of the experiment (17 weeks), the remaining one for 16 weeks. During the course of the experiment, seven quarters developed a haemolytic staphylococcal infection of a temporary nature, whilst in six others infection persisted until the end of the experiment, at which time, eleven quarters in all were still infected.

Mixed Str. agalactiae and Haemolytic staphylococcal infection. At the termination of the experiment, four of the eleven quarters showing haemolytic staphylococcal infection were also infected with *Str. agalactiae.* In two of these cases *Str. agalactiae* preceded and in the other two, followed the commencement of the haemolytic staphylococcal infection.

Str. uberis infection. Before the experiment began one quarter of one cow (756) in Group II was regularly excreting *Str. uberis.* This infection was uncomplicated by other streptococci or haemolytic staphylococci and lasted six weeks when a spontaneous cure resulted.

In the case of one quarter of a heifer (968) in Group I, *Str. uberis* infection developed within the first week of the experiment

and persisted regularly throughout the 17 weeks. This quarter excreted no other streptococcus or haemolytic staphylococcus.

Relation of clots to infection

At the end of the experiment there were 23 *Str. agalactiae* infected quarters uncomplicated by haemolytic staphylococci. Six quarters which never showed clots were infected for an average period of five weeks. Seventeen quarters (74 per cent.) showed clots. In these, *Str. agalactiae* was present for an average duration of 6·5 weeks. The average interval between commencement of infection and the first appearance of clots was 2·3 weeks. Clots thereafter were usually present, being seen during an average of 3·3 weeks out of the remaining 4·2 weeks of infection. Seven quarters were infected with haemolytic staphylococci in pure culture. Of these, three quarters showed clots during an average of one week, with an average period of infection of 13·6 weeks. During the six weeks when *Str. uberis* was present in one quarter of a Group II cow, clots were present for two weeks. In the case of the other *Str. uberis* quarter (in a Group I heifer) no clots were detected during the 17 weeks that this streptococcus was present.

Relation of the Enrichment Technique (incubation of the milk sample) to the detection of Str. agalactiae infection

Of the 27 *Str. agalactiae* infected quarters, eleven showed infection by the enrichment technique on an average of 1·45 days before the streptococci were found on direct plates, in three the direct plate examination was positive on an average of 1·66 days before the infection was found by the enrichment method and in the remaining 13 the two methods gave coincident results.

The above statements refer to animals in which infection became established. In addition to these, there were 175 enrichment positives which apparently had no relation to established infection; they formed 4·7 per cent. of the total 3,700 tests concerned and are regarded as false positives, due either to contamination of the sample from the outside of the teat during sampling or to infection which never became established. It is thought that the former was usually the case, since nearly all occurred during the last four weeks of the experiment when the donor milk was of a viscous nature. In a few cases towards the end of the last week of the experiment it was not possible to be certain whether an enrichment positive was the precursor of an established infection, but this is considered unlikely, especially since examinations were continued for a few more days without any change occurring. In all cases except one (964) there was no difficulty in deciding the date each animal became infected.

Persistence of infection on the outside of the udder

This work was commenced in the eleventh week and from this date until the end of the experiment, *Str. agalactiae* could be generally recovered at any time between milkings from swabs taken from the skin of the teats. Cultural and serological examination of these strains showed them to be identical with the strain excreted by the donor cow. It seems certain that they came from the surface of the udder because, although it would be possible for aerial contamination to occur, regular experiments showed that exposure of swabs to air for five minutes failed to yield *Str. agalactiae.*

At the time when these swabbing experiments were commenced, it had already become evident that certain cows were more resistant to *Str. agalactiae* than others. Efforts were therefore made to determine whether these cows were able to limit the time of survival of *Str. agalactiae* on their teats following the smearing of infected milk, and so prevent these organisms gaining access to the inside of the udder. Three methods were examined:

(1) Swabs were taken daily for five days from the teats of all cows before and after the normal milking procedure, *i.e.*, milking including smearing with infected milk.

The results showed that in a total of 85 post-milking swabs only one failed to yield *Str. agalactiae,* whereas in an equal number of pre-milking swabs, 19 failed to yield this organism. The recovery of *Str. agalactiae* from the pre-milking swabs was, however, made more difficult by the fact that the udders and teats of some cows were splashed with faeces and were not washed before swabbing. Five cows (767, 966, 758, 965, 964) failed to show *Str. agalactiae* on two or three occasions out of the five daily tests. Only one of these cows (965) was not infected with *Str. agalactiae* at the time and she remained free from infection for the duration of the experiment. All the remaining 13 cows showed positive pre-milking teat swabs on at least four occasions out of the five daily tests.

(2) Cows were milked normally in the morning, infected milk was then smeared on to the teats of all cows and teat swabs were taken on seven occasions during the subsequent twelve hours. Of a total of 119 swabs taken, all showed *Str. agalactiae.*

(3) Cows were milked by the normal procedure, *i.e.*, washing of udder, smearing with infected milk and then milking, and swabs were taken from all cows on five occasions during the eight hour interval between morning and afternoon milking. Of the 85 swabs taken 46 were positive ; examination of the remainder was interfered with by contamination derived from faecal splashing of the udder and they may or may not have contained *Str. agalactiae.* All cows, however, were positive at several tests with the exception of 756

which was a moderately susceptible cow as shown by the fact that she became infected a week or two later.

To summarise, these three experiments provided no indication that non-infected cows were able to remove *Str. agalactiae* from the skin of the teat during the interval between milkings better than those which had become infected.

DISCUSSION

The experiments described have shown that dipping the hands once in infected secretion, smearing over the teats and lower parts of the udder and then milking, is a satisfactory method of infection and of testing the relative susceptibility of cows to *Str. agalactiae*. The exposure was more severe than would be the case in normal practice since the neglect of sanitary measures was pushed to the extreme. This severity has, however, served to emphasise the second object of our work, *viz.*, the difference in susceptibility of cows, whilst it leaves no doubt that failure to take the ordinary sanitary precautions is likely to lead to much spread of infection.

It is recognised that only a proportion of animals of a given age become infected: in a herd, this may be largely due to chance. It seems unlikely, however, that in the experiment just described chance could have played an important part, since all the cows received equal treatment, namely, exposure to heavy infection twice daily for 15½ weeks. There seems little doubt, therefore, that some cows are in fact more susceptible than others.

Five of the six older cows in their fourth to seventh lactation had been previously infected with *Str. agalactiae*. It was clearly demonstrated that the current infection was not a breakdown of the treatment by the fact that the previous infection in these older cows was type Ib *Str. agalactiae*, while the new infection produced was IIIa of identical type and colonial appearance with that excreted by the donor cow. These five old cows were originally chosen for this experiment because, on the basis of their previous infection, they were obviously susceptible animals. It is unfortunate that since they were older cows it is not possible to conclude with any assurance which played the greater part, an inherent susceptibility or age.

The fact that the older cows showed the greatest susceptibility and the heifers the least whilst the second calvers were intermediate, suggests that age was an important factor; this contention receives some support from the observation that one old cow (964) whose early history is not known was susceptible, although she had shown no established infection in her previous two lactations. On the other hand, the difference between the heifers and second calvers was small until the 13th week (only one having previously become infected) and was never so marked as between them and the older

previously infected and cured cows. It is possible, therefore, that the susceptibility of the older cows was due to something other than age as, for example, an inherent susceptibility due to some other factor which also accounted for the infection in their earlier lactations: alternatively, it is possible that one infection sensitises or does some damage which predisposes to another (Jones and Little, 1934).

It is not clear what caused the sudden increase in the infection rate which occurred in the 13th week in the heifers and second calvers and, in respect of quarters, also amongst the older cows. It may have been associated with the change of donor during the eleventh week. The original donor gave a secretion which was more or less normal but contained small clots, whilst the second donor had a very clotted and viscous secretion containing *Str. agalactiae* in larger numbers. If the increased infection was associated with the change of donor then it is interesting to note that there was a lag period of 14 days between change of donor and the appearance of this increased infection rate.

It has been suggested that so-called non-specific mastitis may influence the susceptibility of quarters to subsequent *Str. agalactiae* infection (Peterson and Hastings, 1939). This aspect will be dealt with more fully at a future date. It is sufficient to say here that in the work now reported a somewhat larger proportion of quarters which, in the absence of pathogenic organisms, showed abnormality to indirect tests, *i.e.*, electro-conductivity, Whiteside readings and presence of clots, became infected with *Str. agalactiae,* as compared with the proportion showing no such abnormality. This might be taken as evidence in support of the suggestion put forward by Peterson and Hastings. It should be pointed out, however, that in the present experiment most of the quarters which showed evidence of "non-specific mastitis" and subsequently became infected with *Str. agalactiae,* were in the older cows (Group III) and that all of the few quarters in this group which showed no evidence of abnormality also became infected with *Str. agalactia.* It has also been suggested that other infections may predispose to *Str. agalactiae.* This receives no support as regards haemolytic staphylococci or *Str. uberis.*

One other point which deserves emphasis is that something more than infection was produced. The degree of clinical mastitis, including influence on milk production, induration and clots was at least equal to that found in the average heavily infected herd and more than in most: moreover it had been produced in 15 weeks.

SUMMARY

Milk infected with *Str. agalactiae* type IIIa was smeared on to the teats of a herd of 18 cows immediately before each milking, for a period of 15 weeks. During this time bacteriological and

other examinations were made five times weekly. The herd consisted of seven first calved heifers, five second calvers, none of which had previously shown *Str. agalactiae,* and six older cows, five of which had previously shown infection with *Str. agalactia* and had been cured.

Twenty-seven quarters of twelve cows became infected and by the twelfth to 15th week the clinical and other effects simulated those of a severe outbreak of *Str. agalactiae* mastitis in the field.

All the older cows readily became infected with the *Str. agalactiae* present in the secretion used. The first calf heifers were relatively resistant, although towards the end of the experiment two became infected. The second calvers were intermediate in resistance. The difference in susceptibility or resistance was shown in respect of time and of the proportions of cows and quarters infected.

Results indicated that *Str. agalactiae* persisted equally well on the outside of the teats of susceptible and resistant cows.

The question is discussed whether the greater susceptibility of the older cows was a function of their age or due to an inherent susceptibility which accounted for their infection in the previous one or two lactations.

Data are provided to show the relation between infection and appearance of clots and the relation between results of direct plating and enrichment.

The association of previous infection with other organisms or of non-specific abnormalities with susceptibility to subsequent *Str. agalactiae* infection is discussed.

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SOME FACTORS WHICH MAY BE CONCERNED IN THE SUSCEPTIBILITY OF THE BOVINE UDDER TO *STR. AGALACTIAE* INFECTION

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INTRODUCTION

In a previous paper (Lancaster and Stuart, 1949) an experiment was described in which some quarters of some cows were easily infected with *Str. agalactiae* by milking with infected hands, whilst others resisted attempts to infect them for longer periods or for the duration of the experiment which lasted 15 to 16 weeks: no consideration was given to the factors which might be involved, except age and previous infection. In this paper observations will be reported on the relation to susceptibility or resistance of teat injuries, patency of teat ducts, other illness, the possibility of a virus being concerned and the overall importance of the teat barrier as compared with factors beyond it and within the udder. The assessment of the last mentioned was attempted by the sterilisation of the infection produced by milking with infected hands, and the subsequent infusion of small doses of *Str. agalactiae* so as to by-pass the teat sphincter.

CORRELATION OF TEAT INJURIES WITH INFECTION OF QUARTERS

Throughout the experiment on milking with infected hands, involving 18 cows, the presence of injuries to the teats including the smallest superficial sores was recorded. The majority of " injuries " were small superficial sores but deep fissures (chapped teats) were also prevalent at some periods. In only one case was injury attributable to a tread or other trauma. Special note was made of injuries (sores) involving the teat orifice.

It was found that small superficial sores on the teats had no influence on subsequent infection ; in fact a smaller percentage of quarters showing such sores became infected than of those where none was present. The teats of 42 quarters were either completely free from sores or had very small superficial sores for short periods during the experiment. Thirteen (31 per cent.) of those quarters became infected. The teats of 23 quarters had deeper sores or fissures for long periods. After these were present, eleven (48 per cent.) quarters became infected. Sores involved the teat orifice of four quarters ; three of those became infected.

CORRELATION BETWEEN TEAT PATENCY AND SUSCEPTIBILITY OF QUARTERS

At the termination of the experiment on milking with infected hands an attempt was made to estimate teat patency according to the method described by Murphy (1944).

In the twelve first and second lactation cows there was a distinct difference in this respect between some cows and others but no difference could be appreciated between teats of any individual cow. Three of these twelve animals were considered to have patent teats, one of which leaked milk before each milking throughout the experiment while the other two did so at the beginning of their lactations : six of their twelve quarters became infected by milking with infected hands. Eight of these twelve cows were considered to have non-patent teats ; three of their 32 quarters became infected. The remaining cow had constricted teat sphincters ; none of her quarters became infected. One of the six old cows had very constricted teat sphincters ; all four quarters became infected. Difficulty was experienced in deciding whether to classify the other five as having patent or normal teats. Thirteen of their 17 quarters became infected and no difference in patency could be detected between the quarters which became infected and those which resisted.

THE EFFECT OF OTHER ILLNESS ON SUSCEPTIBILITY TO INFECTION

During this experiment all except two cows remained in good general health. One heifer in the eighth week of the experiment on milking with infected hands, developed an acute and severe impaction of the rumen which resulted in almost complete cessation of milk secretion and in inappetence for one week, after which recovery took place and milk yield was gradually restored to almost its former level. Another heifer developed foul of the foot which resulted in serious loss of bodily condition and milk yield from the tenth week of the experiment until its termination. Neither of those two heifers became infected with *Str. agalactiae.*

THE INFUSION OF STERILE MASTITIS SECRETION AND FILTRATES

Secretion from a case of acute mastitis in the field which was found to be sterile on bacteriological examination, was infused into two quarters of a Devon cow in late lactation and free from udder infection. The doses were 1 c.c. and 10 c.c. Two days later, these two quarters gave a thick purulent secretion without signs of acute inflammation. One c.c. of this secretion was infused into one quarter of a heifer in the experiment on milking with infected hands and 1 c.c. of a Seitz E.K. filtrate into another quarter of the same heifer. The experiment was at this time in its fifth week. Three weeks later the secretion from the Devon cow which was still thick and purulent was emulsified in saline. One c.c. of filtrate was infused into one quarter of a second heifer in the transmission experiment. These infusions in the heifers produced no clinical change and the animals did not become infected with *Str. agalactiae* after seven to ten weeks of exposure. The only organisms isolated from milk samples of all three cows considered here were small numbers of non-haemolytic staphylococci or of udder diphtheroids.

Five weeks after the Devon cow had been infused with the sterile mastitis secretion one of the infused quarters became acutely inflamed. The teat especially was much enlarged and no secretion could be withdrawn. She was slaughtered at this time. Lesions which were confined to the teat cistern consisted of haemorrhages on the epithelium and underlying tissues. Microscopic examination and cultures incubated aerobically, anaerobically and in 10 per cent. carbon dioxide revealed no organisms other than a very few non-haemolytic staphylococci. Material taken from the inflamed areas was emulsified in milk from a first lactation Ayrshire heifer free from udder infection and not included in other experiments. The emulsion was filtered through a gradacol membrane and 1 c.c. was infused into two quarters of the heifer. No change occurred in these quarters during an observation period of six weeks. Electroconductivity estimations and modified Whiteside tests (Murphy and Hanson, 1941 ; Murphy, 1942) were carried out on quarter milk samples from the three heifers infused with secretions or filtrates. The milk samples from infused quarters gave weakly positive results on the day subsequent to infusion but were thereafter consistently negative to these tests.

IMPORTANCE OF THE TEAT BARRIER

Treatment of Cows

All quarters of 20 cows were infused with penicillin in aqueous solution. Seventeen had been exposed to infection by milking with infected hands, two were infected under natural conditions in the field and one was a non-infected Jersey heifer in her first lactation.

Twenty-seven quarters were infected with *Str. agalactiae* at the time of treatment ; eight had been infected for more than three months and five for more than two months. Eleven quarters were infected with haemolytic staphylococci and one with *Str. uberis*. Twenty-nine quarters, mostly *Str. agalactiae* quarters, had given clinically abnormal milk at some time during the previous four months. *Str. agalactiae* could be isolated readily from the skin of the udder and other parts of the cows and from almost all parts of the cowshed. Details of this work will be published later.

The dosage of penicillin used was 100,000 units dissolved in 50 c.c. of distilled water infused into each quarter daily after the afternoon milking for five days. On the second and fifth days of treatment the animals were thoroughly sprayed with hypochlorite solution (3 oz. " Chloros " to one gallon warm water). On the fifth day the cowshed, tools and milking utensils were disinfected with a 1 per cent. cresol solution. Penicillin cream containing 1,000 units per g. was rubbed on the teats and milkers' hands at every milking during treatment and for three days afterwards.

Quarter milk samples were examined bacteriologically 14 times during 26 days following treatment. No *Str. agalactiae* and no *Str. uberis* was found by direct plating of 0·1 c.c. on the surface of sheep blood agar or by enrichment technique. Very small numbers of haemolytic staphylococci were, however, present on five of the blood agar plates which were examined during this period and numbered more than 1,000. Most quarters were freed from infection. After the completion of treatment and disinfection, attempts to recover *Str. agalactiae* from the teats and surface of the udders and from all parts of the cowshed gave uniformly negative results.

Attempts to Estimate a Minimal Infective Dose of Mastitis Secretion. (Preliminary Experiment)

As quarters which had remained free from infection in the experiment on transmission with infected hands were more numerous than those which became infected, it was decided to make a preliminary experiment on a few cows to attempt to find the largest dose of mastitis secretion which, when injected *via* the teat canal, would fail to infect such apparently resistant quarters. This dose would then be injected into all quarters of the remaining cows to see whether there would be any difference in the infection rates in quarters classed as resistant or susceptible in the experiment on infection by milking with infected hands.

For this purpose two cows, in which all the quarters had resisted infection in the transmission experiment and the Jersey heifer mentioned above, were moved after treatment to separate clean premises. Quarter milk samples were examined bacteriologically eight times during 13 days following treatment. No streptococci or staphylococci were present.

The mastitis secretion used for infusion was a very clotted, viscid sample from the donor used in the latter part of the previous experiment (928). Immediately after it was taken the sample was mechanically shaken in a bottle with glass balls for 50 minutes, after which a viable count of *Str. agalactiae* was carried out in triplicate in deep blood agar. This gave a reading of approximately 2,000,000 *Str. agalactiae* per c.c. The sample was kept in cold store overnight and the following day a second viable count was carried out with approximately the same result. This procedure was closely followed on all samples for subsequent infusions of quarters so that the numbers of *Str. agalactiae* injected were known before infusions of quarters were made. Quarter milk samples of the three cows under test were taken with sterile precautions. Dilutions of the donor's secretion were made up separately with these quarter samples so that each quarter was infused with a dilution made up in its own milk. The time taken between making up of

dilutions and the infusion of these into the quarters was less than one hour.

The ends of the teats were cleaned with sterile water and sterile cotton wool. Infusions were carried out by means of syringes and smooth narrow-bore syphons, care being taken not to injure the teat ducts. Doses ranged from 1 c.c. of a 1 : 1,000 dilution up to 5 c.c. of undiluted secretion. Infusions were made after the afternoon milking and all subsequent milkings were carried out normally. This procedure was followed in subsequent infusions.

TABLE I

RESULTS OF INFUSION OF INCREASING DOSES OF *Str. agalactiae* MASTITIS SECRETION INTO CLEAN ANIMALS

Cow	*Quarter*	*Doses of secretion*	*Duration of infection*
963	LF	1 c.c. of 1 : 1,000 dilution	0 days
967	LF	1 : 300 „	5 „
988	LF	1 : 100 „	6 weeks
963	LH	1 : 50 „	16 days
967	LH	1 : 25 „	9 „
988	LH	1 : 12 „	6 weeks
963	RF	1 : 6 „	3 days
967	RF	1 : 3 „	6 weeks
988	RF	1 c.c. undiluted	5 „
988	RH	2 c.c. „	2 days
967	RH	3 c.c. „	0 „
963	RH	5 c.c. „	0 „

Quarter milk samples were examined bacteriologically five days each week following infusion. *Str. agalactiae* were recovered from nine of the twelve quarters for periods varying from two days up to the end of the six weeks period of observation. Details are given in Table I. A serious mastitis developed in three of the quarters of one cow in the third and fourth weeks after infection, leading to complete loss of milk secretion in a few weeks. Clots were present in the milk of two other quarters for shorter periods while they were infected.

The quarters which received the highest doses, namely 2 c.c., 3 c.c. and 5 c.c. of undiluted secretion, had clots in the foremilk consistently over the six weeks of observation. The only organisms present were udder diphtheroids in small or moderate numbers. As the failure of large doses of secretion to infect these quarters was difficult to understand, an Ayrshire heifer (991) in her first lactation and free from udder infection was, at a later date, infused in three quarters with secretion from the same donor cow (928) in doses of 1 c.c. of a 1 : 100 dilution, 1 c.c. of a 1 : 10 dilution and 3 c.c. of undiluted secretion, respectively. These quarters excreted *Str.* agalactiae for 0, 5 and 1 days respectively. Later a Friesian heifer (969) which had resisted *Str. agalactiae* infection in the transmission

experiment was infused in three quarters with a similar secretion but from a different donor cow (988) in doses of 1 c.c., 2 c.c. and 10 c.c. of undiluted secretion respectively. *Str. agalactiae* was recovered from those quarters for 0, 2 and 5 days respectively.

From these preliminary experiments it seemed that there was no definite connection between the dose and the establishment of persisting infections. It was thought that a dose equivalent to the 1 c.c. of a 1 : 1,000 dilution of secretion, *i.e.*, containing 2,000 *Str. agalactiae*, might infect susceptible quarters and be a suitable dose for distinguishing such quarters from others.

Infusion of Quarters with Equal Doses of Mastitis Secretion. (Main Experiment)

Twenty-six days after the penicillin treatment all quarters of 16 cows were infused with 1 c.c. of a 1 : 4,000 dilution of mastitis secretion from donor (928) having a viable count of approximately 2,000 *Str. agalactiae*. Dilutions were made up in bulk milk from each cow separately and infusions carried out within one hour. Viable counts of each dilution, made after infusion, did not vary greatly. The infusions were carried out after the afternoon milking, in the manner already described and subsequent milkings were carried out normally.

Quarter milk samples were daily examined bacteriologically. *Str. agalactiae* were recovered from only four quarters and in two of these they were absent after the first day. A week later all quarters were again infused with 1 c.c. of a 1 : 300 dilution of secretion from donor (928) containing approximately 20,000 *Str. agalactiae* and diluted in each cow's own milk as above. This dose resulted in a further nine quarters excreting *Str. agalactiae*, five of which became free after five days. Of the six quarters which showed infection beyond this period, four had become infected in the experiment on transmission by milking with infected hands. One week following the second infusion a third was carried out. At this time the viable count of *Str. agalactiae* in the secretion of donor (928) had fallen to less than 1,000 per c.c. Secretion from another cow (988), a purulent viscid sample with a viable count of approximately 100,000 *Str. agalactiae* per c.c., was used. One c.c. of this secretion was infused into all quarters and bacteriological examinations of quarter milk samples were carried out for 18 days after which all cows were again treated. In the examinations referred to, *Str. agalactiae* was recovered from 36 quarters. The correlation between these results and those obtained by milking with infected hands in the 14 cows under test in both experiments is as follows:

In the experiment on milking with infected hands, nine quarters (eight in the old cows and one in a second lactation cow) became infected within seven weeks, 14 quarters became infected after ten

weeks, while 30 were not infected at the end of the experiment. These groups will now be referred to as " susceptible," " moderately susceptible " and " resistant" respectively.

Injection of mastitis secretion *via* the teat canal resulted in all nine of the " susceptible " quarters excreting *Str. agalactiae*, eight (88 per cent.) of them in large numbers, up to the 18th day. The remaining quarter cleared itself of infection after one day ; this quarter, though infected early in the previous experiment, had excreted streptococci in small numbers irregularly while all the other " susceptible " quarters had been solidly infected. Eight of the 14 " moderately susceptible " quarters excreted the streptococci after infusion, four (28·6 per cent.) up to the end of the observation period and four for less than one week. Fourteen of the 30 " resistant " quarters excreted *Str. agalactiae* after infusion, four (13·3 per cent.) up to the end of the observation period and ten for less than one week. In three of the four quarters showing the most persistent infections the numbers of streptococci were small compared with those in infected quarters in the " susceptible " and " moderately susceptible " groups.

Two cows infused with mastitis secretion had not been included in the experiment on milking with infected hands but had become naturally infected with *Str. agalactiae* in all eight quarters before they were purchased. Two quarters of one cow were completely fibrosed and non-functional. Following infusion with secretion five out of the six secreting quarters became re-infected and remained so over the period of observation. The remaining secreting quarter and the two non-functional quarters did not become infected. The donor cows of the first and second experiment were infected one from the other so that all infections were with the same strain of *Str. agalactiae*. The serological type of *Str. agalactiae* isolated from every quarter which became infected in both experiments was checked by agglutination and agglutination-absorption tests and was found to be the same as that of the original donor, *viz.*, type 3a (Stableforth, 1932, 1937).

DISCUSSION

In this attempt to estimate the relative importance of factors influencing the susceptibility of quarters of the bovine udder to experimental infection with *Str. agalactiae*, consideration has been given to the role played by the teat barrier, teat injuries and to the effect of infusion of filtrates of mastitis secretions. Previous workers have shown that surgical impairment of the teat sphincter was followed by infection of quarters by streptococci when cultures were applied externally while a similar application to intact teats did not produce infection (Christiansen and Nielsen, 1934 : Bendixen, 1934). The latter author (1933, 1935) also states that in the field almost all quarters with teat ends showing cicatrices were infected

with streptococci, 85 per cent. of which were *Str. agalactiae*. The importance of trauma in infection is also borne out by the figures given by Ferguson (1944). Eighty-nine per cent. of injured quarters were found to be infected compared with 16 per cent. of uninjured quarters.

The correlation of teat patency with susceptibility to infection has not been definitely established. Murphy (1944) has pointed out the difficulties encountered. His results show a tendency towards a greater percentage of infection in patent quarters especially when compared with those consistently non-patent over a number of tests. Little (1937b) reported that two old cows with easily milked teats were more readily infected experimentally with *Str. agalactiae* than were heifers with non-patent teats. Bull *et al.* (1940) and Murnane (1941) found no correlation between teat patency and the incidence of mastitis.

The possibility of a filtrable virus being the primary infective agent in mastitis has been postulated several times and the work of Broadhurst *et al.* (1939), Petersen, *et al.* (1938, 1939), Hastings *et al.* (1937, 1939, 1940, 1943) and Little and Plastridge (1946) has lent some support to this theory. Nyiredy (1941), however, failed to set up mastitis by infusion of mastitis secretion filtrates into the udder; whilst Stableforth (private communication) has on many occasions attempted without success to set up mastitis with filtrates or secretions containing no recognised pathogens. Jones and Little (1934) reported that the infusion of mastitis secretion filtrate in one cow followed by infusion of streptococcal culture failed to produce infection.

An attempt has been made to correlate susceptibility of quarters to infusion of *Str. agalactiae* beyond the teat barrier with that shown earlier (Lancaster and Stuart, 1949), towards infection by application to the outside of the teats and udder. The validity of the conclusions reached must depend on whether the infections produced by external application were in fact sterilised. It is believed that they were because repeated cultural examination, including enrichment tests, failed to show any infection at any time and because further similar treatments of these cows at the conclusion of this transmission experiment and of a herd of 52 cows of which 25 were infected with *Str. agalactiae,* have shown that almost all infections were sterilised, quarter milk samples giving negative results to repeated cultural examinations during a period of more than 18 months.

The fact that quarters which were susceptible, moderately susceptible or resistant to hand infection also showed corresponding degrees of susceptibility or resistance to infusion past the teat barrier suggests that the main defence mechanisms are within the udder rather than in a teat barrier, and would contradict the correlation of susceptibility to teat patency which apparently existed in the younger

cows. It is possible that the primary infection caused changes within the udder which influenced the susceptibility to subsequent infection by infusion. This suggested change would more probably be of a pathological nature than a sensitisation, as all quarters had three weekly infusions which should, to some degree at least, have sensitised them before the final dose. Infections of three months or longer produced no immunity, but rather an increased susceptibility to the same strain of *Str. agalactiae*.

As mastitis secretion was used throughout to produce infections, all quarters would have received the same inoculum of virus had it been present. Whereas there is little or no evidence in support of the theory of a virus being the primary infective agent of mastitis in this work, the infusions of sterile secretions and filtrates were too limited to disprove it. An acute mastitis was set up in an udder infused with sterile mastitis secretion and a slight but persistent clinical change without infection, other than by small numbers of udder diphtheroids, was brought about by the infusion of undiluted *Str. agalactiae* mastitis secretion in three other cows. Attempts to transmit the condition from the acute case were, however, unsuccessful and the infusions of the secretion and its filtrates into quarters of two heifers did not make them susceptible to *Str. agalactiae* infection. It is possible that the reactions referred to in quarters infused with secretions were due solely to their being foreign material. No explanation can be given for the persistence of the reactions.

In attempts to find a minimal infective dose of mastitis secretion no simple correlation was found between the dose and establishment of infection. This is contrary to the finding of Little (1937a) using dilutions of cultures. Rienhoff (1929), however, reported that doses of up to 50 c.c. of mastitis secretion failed to infect some quarters, while 6 c.c. set up mastitis in another. The reaction of the udder to undiluted secretion may be the reason for the failure of these large doses to produce infection, but the susceptibility of quarters probably plays an important part.

It is not considered that the presence of injuries to the teats was of great importance in influencing susceptibility in the experiment on milking with infected hands although a somewhat greater percentage of quarters, the teats of which had deep fissures, did become infected compared with those which had no or only small superficial sores. The effect of injury involving the teat orifice cannot be estimated accurately as the numbers were small and whereas three out of the four quarters with such sores became infected, these three quarters proved equally susceptible to infection by external application of *Str. agalactiae* at a later date when no sores were present. This conclusion, however, is not in conflict with

those of Ferguson and others who have shown the importance of trauma, as only one injury in this experiment was of traumatic origin. Bull *et al.* (1940) found that an outbreak of sores on the teats did not influence the incidence of mastitis in their experimental herd.

SUMMARY

Fourteen cows, the quarters of which had been classed as susceptible, moderately susceptible or resistant as a result of attempts to infect them with *Str. agalactiae* by milking with infected hands, were treated with penicillin and freed from infection. They were then injected with *Str. agalactiae via* the teat canal in order to see whether susceptibility or resistance to this route of infection was correlated with that shown to hand infection in the previous experiment.

Quarters which were most susceptible to infection by external application were correspondingly susceptible to infection by infusion. Infection also followed the infusion of secretion in two additional cows which had been infected under natural conditions in the field and cured.

In trying to find a suitable dose of mastitis secretion for infection *via* the teat canal it was found that in five cows, whilst doses of intermediate sizes were regularly followed by infection, the duration was variable and often short. Smaller doses failed to infect and larger doses likewise failed or were followed by transient infections.

Attempts were made to set up mastitis by infusion of sterile mastitis secretions and filtrates in four cows. Two of those were subsequently exposed to infection with *Str. agalactiae,* neither becoming infected.

Data are given on the influence of injury and teat patency on susceptibility to experimental infection with *Str. agalactiae.*

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A COMPARISON OF DIFFERENT METHODS OF INOCULATING GUINEA-PIGS WITH THE VIRUS OF FOOT-AND-MOUTH DISEASE*

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INTRODUCTION

Since Waldmann and Pape (1920) reported the successful transmission of foot-and-mouth disease to the guinea-pig by scarification of the hind pads, this animal has been extensively used in laboratory studies of the virus. The site of inoculation most commonly selected is the large, hairless pad of the hind foot, but various methods of introduction of the virus have been described. Stockman, *et al.* (1927) found that infection was more regular following intradermal inoculation than after scarification. They also noted that a reduction in the number of intradermal tracks from three on both hind pads to one on one pad lessened the severity of infection as judged by the subsequent development of secondary lesions. Schmit-Jensen, *et al.* (1936) have expressed preference for a method of multiple puncture of one or both pads. Henderson (1945), in an attempt to increase the number of observations available from each guinea-pig, compared multiple intradermal tracking of both pads with two single tracks on each. He found, however, that fewer guinea-pigs reacted when inoculated with four single tracks compared with multiple tracks.

In visits to other research institutes, the writer has been told that at Pirbright multiple tracking of both pads is the routine method of inoculation of guinea-pigs; at the Vaccine Institute at Basle, Switzerland, four separate tracks are made on one hind pad; and at Buenos Aires multiple tracking of one pad is used. In the absence of the results of comparative tests of the sensitivity of each method, these variations in the method of inoculation make it difficult to compare and repeat experiments from different institutes.

METHODS

Strains of Foot-and-Mouth Disease Virus.—Pirbright strain No. 1 (Vallée O type), Pirbright strain GB (Vallée A type), and Pirbright strain GC (Waldmann C type) were the stock immunological type strains of the Research Institute, and all had been passaged regularly in guinea-pigs for many years. Before each experiment they were passaged three or four times and lymph was collected 24 hours after inoculation from the primary lesions of the last passage.

Pirbright strain No. 643 (Vallée O type) was selected as a strain

* The British Foot-and-Mouth Disease Research Committee kindly extended facilities for carrying out this work at their Research Institute, Pirbright, England, under the general supervision of the Director, Dr. I. A. Galloway.

that had not been adapted to the guinea-pig. It was isolated from an outbreak in swine in Great Britain in 1946 and had been passaged once in cattle.

Preparation of Virus Suspensions and Dilutions.—In every case the infective material used was guinea-pig vesicle lymph diluted 1 in 10 in Hartley's digest broth. After storage overnight at 4° C., it was centrifuged for 25 minutes at 2,500 r.p.m. and the supernatant liquid passed through a 0·6μ APD gradocol membrane. This filtrate was used as the starting point for dilutions prepared in Hartley's broth, *p*H 7·6.

Test Animals and Methods of Inoculation.—The guinea-pigs were animals of the Pirbright Institute stock, weighing 450 to 500 g. The methods of inoculation were (1) multiple intradermal tracking of both pads; (2) two separate intradermal tracks on each pad; (3) one intradermal track on each pad; (4) multiple puncture of one or both pads, 30 punctures per pad followed by gentle rubbing in of the virus suspension; (5) five lines of scarification on each pad followed by gentle rubbing in of the virus suspension. All the intradermal tracks were made with a blunt-pointed needle, the inoculum being injected at the same time. These methods are illustrated in Fig. 1, one foot only being shown.

Following inoculation, the guinea-pigs were observed daily for seven days. In titrations, the 50 per cent. positive end-point was calculated by the method of Reed and Muench (1938).

RESULTS

A comparison was first made between multiple tracking of both pads and multiple puncture of one pad by titration of a filtrate of strain 1. The 50 per cent. positive end-point by multiple tracking was $10^{-5\cdot5}$ and by multiple puncture, $10^{-3\cdot8}$ (Table I). Two groups of eight guinea-pigs were then inoculated with the 10^{-4} dilution of the same filtrate. By multiple tracking all of the test animals reacted with primary and secondary lesions, whereas none of those inoculated by multiple puncture showed any reaction.

In the next experiment five methods were compared by titrating a filtrate of strain GC. The 50 per cent. positive end-points were respectively (1) multiple tracking both pads, $10^{-4\cdot4}$; (2) two tracks on each pad, 10^{-4}; (3) one track on each pad, $10^{-3\cdot8}$; (4) multiple puncture both pads, $10^{-2\cdot6}$; (5) five lines of scarification on each pad, $<10^{-2\cdot2}$ (Table II).

An attempt was made to accentuate any difference that might exist between the various intradermal methods by inoculating three groups of 20 guinea-pigs by multiple tracking, by two tracks and by one track respectively, using the 50 per cent. positive dilution of a filtrate of strain GB previously determined by multiple tracking. The result is shown in Table III, where it will be seen that no significant difference was demonstrated between the three methods. The

TABLE I

TITRATION OF A STRAIN NO. 1 FILTRATE BY MULTIPLE TRACKING AND BY MULTIPLE PUNCTURE. THREE GUINEA-PIGS PER DILUTION

Dilution	*Multiple tracking both pads*		*Multiple puncture one pad*	
	+	—	+	—
10^{-3}	3	0	3	0
10^{-4}	3	0	1	2
10^{-5}	3	0	0	3
10^{-6}	0	3	0	3

TABLE II

TITRATION OF A STRAIN GC FILTRATE BY FIVE METHODS OF INOCULATION OF THE HIND PADS. FOUR GUINEA-PIGS PER DILUTION

Dilution	*Multiple tracking*		*Two tracks each pad*		*One track each pad*		*Multiple puncture*		*Scarification*	
	+	—	+	—	+	—	+	—	+	—
$10^{-2.2}$	–	–	4	0	4	0	4	0	1	3
10^{-3}	4	0	4	0	4	0	0	4	0	4
$10^{-3.8}$	4	0	2	2	2	2	0	4	0	4
$10^{-4.6}$	1	3	1	3	0	4	0	4	0	4
$10^{-5.4}$	0	4	–	–	–	–	–	–	–	–

TABLE III

THREE GROUPS OF TWENTY GUINEA-PIGS INOCULATED WITH THE 50 PER CENT. POSITIVE DILUTION OF A STRAIN GB FILTRATE PREVIOUSLY DETERMINED BY MULTIPLE TRACKING

Dilution	*Multiple tracking*		*Two tracks each pad*		*One track each pad*	
	+	—	+	—	+	—
10^{-5}	9	11	6	14	7	13

TABLE IV

TWO GROUPS OF TWENTY GUINEA-PIGS INOCULATED WITH A STRAIN (643) NOT ADAPTED TO THE GUINEA-PIG

Dilution	*Multiple tracking both pads*		*One track on each pad*	
	+	—	+	—
$10^{-3.3}$	15	5	12	8

TABLE V

TWO GROUPS OF TWENTY GUINEA-PIGS INOCULATED BY ANOTHER WORKER WITH A DILUTION OF A STRAIN NO. 1 FILTRATE

Dilution	*Multiple tracking both pads*		*One track on each pad*	
	+	—	+	—
$10^{-4.6}$	14	6	10	10

amount of generalisation that followed these primary reactions was also the same in each group.

A comparison between multiple tracking and single tracking was further made by using strain 643, which had not been adapted to the guinea-pig. Since personal differences in technique might affect the results, inoculations were also made by an independent worker. The results of the two experiments are shown in Tables IV and V. Although there was a tendency for multiple tracking to produce quicker and more extensive reactions, and although the number of reactors by this method was always higher than by single tracking, the difference was not great enough to be significant.

In the comparisons shown in Table II, multiple puncture and scarification were both much less sensitive methods than any method using intradermal tracking. One experiment has been done to determine the extent of any difference that might exist between puncture and scarification. A filtrate of strain GB having an end-point by multiple tracking of 10^{-5}, was titrated by multiple puncture on both pads and by five lines of scarification on each pad. The multiple puncture end-point was $10^{-3.5}$ and the scarification end-point was $10^{-2.5}$, a result which, although not showing a great difference between puncture and scarification, again emphasises the greater sensitivity of multiple tracking.

DISCUSSION

The results of these experiments show that considerable variation exists in the reaction of the guinea-pig to the virus of foot-and-mouth disease depending on the method of inoculation of one particular site, namely, the dermis of the plantar pads. Introduction of the virus suspension into the thickness of the dermal tissue by intradermal tracking proved to be far superior for detection of minimal quantities of virus than either superficial puncture or scarification of the same area. No significant difference was demonstrated between one intradermal inoculation and multiple tracking of each pad, although in every case more reactors occurred among the animals inoculated by multiple tracking ; moreover, in general, the reaction was quicker in developing and tended to be more extensive. These results confirm observations made by Henderson (1945). The conclusion is reached that multiple intradermal tracking of both pads is the most sensitive of the five methods examined of inoculating the guinea-pig for detection of the virus of foot-and-mouth disease.

SUMMARY

Comparative tests of different methods of inoculating the hind pads of the guinea-pig with the virus of foot-and-mouth disease are described.

Intradermal tracking was much more sensitive for detection of virus than either multiple puncture or scarification, the end-point

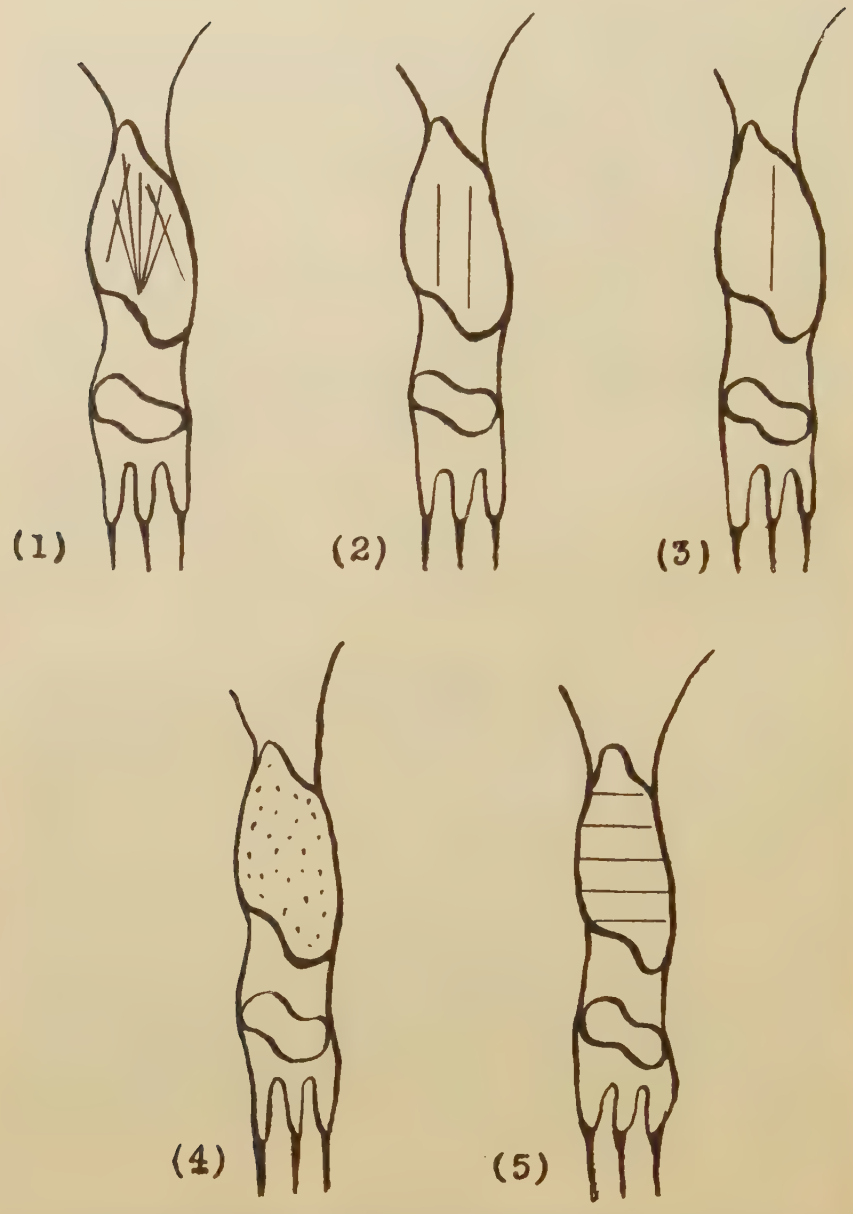

FIG. 1.—Methods of inoculation of the hind pads of the guinea-pig. (1) Multiple intradermal tracking; (2) two separate intradermal tracks; (3) one intradermal track; (4) multiple puncture; (5) five lines of scarification.

in titrations by the latter methods being ten- to one hundredfold lower than by the intradermal methods.

The conclusion was reached that, of the methods examined, multiple tracking of both hind pads is the most reliable method of intradermal inoculation of the guinea-pig.

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EXPERIMENTAL USE OF DIMIDIUM BROMIDE (1553) IN THE TREATMENT OF *T. CONGOLENSE* INFECTION IN ZEBU CATTLE

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INTRODUCTION

Since its introduction in 1944, Dimidium Bromide (1553), a member of the phenanthridinium group of trypanocides, *viz.*, 2 : 7-diamino-9 phenyl-10 methylphenanthridinium bromide has been tested in East Africa against *Trypanosoma congolense* with varying results.

EXPERIMENTAL WORK

Carmichael and Bell (1944) reported favourably on this drug and ascribed to it high trypanocidal qualities. They stated that it may be administered by subcutaneous injection without causing undue local reaction. They pointed out that it appeared to stimulate a greater local reaction on intramuscular than on subcutaneous injection. The dose used in their experiments varied from 1 to 2 mg. per kg. body weight in 1 per cent. and 2 per cent. solutions, and they found that 1 mg. per kg. body weight gave satisfactory cures. Bell (1945) considered the minimum curative dose to be 0·8 mg. per kg. He reported, however, that out of 27 animals receiving the drug subcutaneously, three developed sloughs. Doses of 3 mg. per kg. and above caused a delayed photosensitisation with high mortality (Bell, 1947). Barnett (1946) reported favourably on the trypanocidal powers of 1553 but described serious local lesions in grade cattle when 0·5 per cent. and 2 per cent. solutions were injected intravenously, intramuscularly and subcutaneously. He also expressed the opinion that the subcutaneous route was to be preferred.

Randall and Beveridge (1946) investigated the toxicity of 1553 and found the toxic level to be between 1 and 2 mg. per kg. body weight. In the light of their findings the original claims made by Carmichael and Bell (1944) and Bell (1945) both as regards therapeutic index and absence of local irritation appear to be over optimistic.

Series I

Early trials with Dimidium Bromide were carried out at this laboratory. Seventeen Zebu oxen naturally infected with *T. congolense* were treated, using doses of 1553 of approximately 1·5 mg. per kg. and 0·75 mg. per kg. body weight in 0·5 per cent. and 0·25 per cent. solution respectively. The drug was given either subcutaneously or intramuscularly into the gluteal mass and in both methods was dispersed by massage.

The trypanocidal effect of the drug was marked. In all cases, whether the larger or smaller dose of the drug was used, trypanosomes had disappeared from the peripheral blood within 24 to 36 hours. More detailed examination of the trypanocidal action was made in seven cases. In two of these, treated with the larger dose,

trypanosomes were present, though reduced in numbers and showing signs of disintegration, four hours after injection. In four others (three treated with the larger and one with the smaller dose), trypanosomes had disappeared from the peripheral circulation by the fourth hour after injection. In one ox, treated with the smaller dose by the intramuscular route, trypanosomes had disappeared even within two hours. Of the 17 oxen treated only two suffered relapses, one after three months and the other after five months. The former had received a smaller dose than the remainder of its group, *viz.*, approximately 0·9 mg. per kg., while the latter had received the drug at the rate of 0·75 mg. per kg. Both these animals recovered after a second dose of the drug.

In this experiment, subcutaneous injection caused marked local swellings which were generally hard, cold and painless and persisted for several weeks. In a few cases a slight loss of hair occurred at the site of injection. Intramuscular injection caused swelling of the gluteal muscles with, in some cases, slight temporary lameness. These muscular swellings subsided after about 14 days.

Series II

As a result of consultations at the Standing Veterinary Research Committee Meeting in February, 1948, a further experiment was planned to be carried out in Kenya, Uganda, Tanganyika, and Northern Rhodesia. In the Tanganyika experiment 57 cattle, infected by syringe from an ox naturally infected with *T. congolense*, were treated with Dimidium Bromide. The animals were divided into groups so that, as far as possible, the three routes of administration were evenly distributed. In addition, the groups were further subdivided according to the dose, *viz.*, 1·0, 1·5 and 2·0 mg. per kg. Variations in the time elapsing between the establishment of infection and treatment were also arranged so that some animals were treated within ten days of showing trypanosomes in the peripheral circulation, some between ten and 20 days and the others between 20 and 30 days. The drug was given in freshly prepared 2 per cent. solutions. Five additional cattle, infected with the same trypanosome, were treated with Phenanthridinium Chloride (897) at the rate of 1·5 mg. per kg.

Blood films from the treated cattle were examined every three days for the first few weeks and thereafter at weekly intervals. Observations were maintained for six months, at the end of which period blood from the groups of treated cattle was bulked and injected into goats known to be free of trypanosomes. In addition, all the cattle were weighed weekly as a guide to general condition.

The results of this experiment were spectacular. In every case, including those treated with 897, complete cure was effected by means of a single injection. Doses at the rate of 1 mg., 1·5 mg., and 2 mg. per kg., irrespective of the route by which they were

given, proved effectively trypanocidal. No relapses occurred. The virulence of the trypanosome was demonstrated by the fact that two of the originally infected animals which were being kept for treatment after 30 days, died of trypanosomiasis before treatment was given.

In many of the treated animals, local reactions occurred at the sites of intramuscular and subcutaneous injection. The intramuscular reaction occurred one or two days after injection in the form of swelling, heat and a degree of lameness. The heat and lameness disappeared after seven to 14 days and the swelling subsided, but in a few animals some signs of induration remained for one or two months afterwards. The subcutaneous reaction developed more slowly and generally a large, hard swelling, in most cases showing little heat and causing no lameness, appeared, often gravitating towards the elbow. The swelling subsided very slowly but a small area of induration usually persisted for two or three months. In a few of the animals a small patch of hair was lost from the area surrounding the site of needle puncture, and in one plaques formed in the skin of the rump and ears. These sloughed off eventually.

Series III

A few animals in a herd consisting of 22 heifers, 23 dry cows and two calves were found to be harbouring *T. congolense*. It was decided to treat the whole herd with Dimidium Bromide at a dose of 1·5 mg. per kg. subcutaneously in 2 per cent. solution. Forty-one days later, six cows showed inappetence, malaise and slight pyrexia. Within a week, four more cows developed the same symptoms. As the condition advanced, hard plaques appeared on the skin of the udders, a high degree of icterus was shown in the membranes and rumination and appetite were diminished. In some of the cows, plaques appeared on the muzzle, face, ears, dewlap, in the axillae and in the groin. In all cases, the plaques sloughed off leaving large areas of flesh exposed: in one cow, half of one of the teats was lost. At the first signs of the disease, palliative treatment was commenced. Injections of calcium borogluconate and glucose-saline were given followed by repeated small doses of calomel and sodium sulphate. The animals were kept in stalls and special attention was paid to their feeding and management.

One of the cows which calved a few days after showing symptoms, died and *post-mortem* examination showed the following: jaundice of the subcutaneous tissues and of all internal organs; large ecchymoses in the dorsal parietal pleurae; ulceration of the tongue; consolidation and, in parts, necrosis of the left lung; emphysema and congestion of the right lung; ecchymoses on the pericardium. The liver was enlarged and icteric; the gall bladder small and its contents congealed; the kidneys were jaundiced. The abomasum which contained sand and partially digested blood had an ulcer

at the pyloric end, while the recently gravid uterus contained necrotic portions of foetal membrane and the uterine mucous membrane was deeply inflamed with areas of necrosis. All the other cows recovered slowly.

The condition described above corresponds in many respects with that described by Bell (1947) as a sequel to Dimidium Bromide treatment. Many of its features suggest photosensitisation but no photophobia was noticed. All the animals affected were cows heavily pregnant and all showed the first signs of the disease within a few weeks of parturition.

Three factors possibly involved in the above experiment are: (*a*) treatment with Dimidium Bromide, (*b*) advanced pregnancy and (*c*) a dietetic factor. In regard to (*c*) the herd had been grazing on a pasture recently reclaimed from bush and containing many weeds, which were profuse and succulent as the time of the year was January and the rains were well established

DISCUSSION

Dimidium Bromide is shown by the experiments of Series I and Series II to be extremely trypanocidal. Doses of 1 mg. per kg. and over effected a cure in Zebu cattle infected with *T. congolense,* after one administration. The drug can be given to cattle subcutaneously and intramuscularly in 2 per cent. solution as well as intravenously. Certain undesirable local effects occur after subcutaneous and, to a lesser extent, intramuscular injection and these, although not serious, might detract from the value of a beast if butchered within a month or two of treatment. The most satisfactory and convenient method of administration in the field is intramuscular injection.

In several cases, the drug was given at the rate of 2 mg. per kg. without untoward sequelae. In one case in Series II an ox receiving a dose of 1 mg. per kg. showed a mild manifestation of the skin condition which has been likened to photosensitisation. In Series III, the delayed toxic effects were complicated by at least two other factors, *e.g.,* pregnancy and possible plant poisoning. The affected cattle received doses of 1·5 mg. per kg. only.

These experiments, therefore, afford confirmatory evidence that Dimidium Bromide treatment is attended by the dangers of delayed toxic effects. The facts do not establish this type of toxicity as due to the drug *per se* but suggest that its use might be contributory to such toxic phenomena. The fact that the symptoms described in Series III appeared between 40 and 50 days after treatment with Dimidium Bromide, suggests a sequence of toxic effects, leading to the final manifestation, similar to that described by Bell (1947) and Randall and Beveridge (1946). Many of the cattle in Series III had patches of white hair on their bodies, but most of these were over black skin and were unaffected. Other white haired

areas, as, for example, a white face in one cow and a white dewlap in another were over skin which was less deeply pigmented and these areas were seriously involved. It appears, therefore, that the condition was definitely a form of photosensitisation and the invariable involvement of the udder was undoubtedly due to the paucity of pigmentation in the skin of this organ, rendered of even less protective value by the physiological distension of lactogenesis in pregnant cows. The mildness of symptoms, the absence of photophobia and the high recovery rate were probably due to the heavy skin pigmentation which was a characteristic of all the affected cows. It is possible that the drug Dimidium Bromide may have an effect on the function of a part or parts of the body, which, if severe enough, can interfere with the metabolism of photodynamic substances and bring about an increase in the photosensitivity of the skin.

It has been shown by Quinn (1933) that by raising the blood level of photosensitising substances such as haematoporphyrin and certain fluorescent dyes, the symptoms of photosensitisation can be induced without concurrent liver damage and icterus. This suggests, then, that in the affected animals in Series III, the rise in blood level of these substances was a consequence of the damage, perhaps to the liver, caused by Dimidium Bromide at a season of the year when an abundance of chlorophyll and other photodynamic substances was present in the herbage consumed by the animals.

These experiments confirm the results of earlier work in demonstrating that Dimidium Bromide is a most powerful trypanocide. They have also confirmed previous views that its use under certain circumstances may result in undesirable effects. Considering the small numbers of cattle treated with Dimidium Bromide which have shown these effects, in comparison with the large numbers which have been treated in these and other experiments, it is obvious that the conditions which assist in bringing about these effects, are limited. It would, therefore, be regrettable if such limited conditions should militate against the use of a drug which, otherwise, would form a most important weapon against cattle trypanosomiasis. Work is, therefore, required on this aspect of the problem and it is hoped that it will be carried on at this laboratory. In the meantime, it is difficult to define the limits between minimum therapeutic dose and maximum tolerated dose. It is probably safe to assume that for field treatment, a dose of 1 mg. per kg. will be satisfactory provided careful observation is maintained for the first signs of delayed toxic phenomena which may develop into photosensitisation. It must be remembered, however, that the solubility is not a constant factor and this may account for the varying reports regarding local reactions after the injection of the drug. As changes do take place in solutions of Dimidium Bromide (Wilde and Beakbane, 1948) these should be made up freshly for the treatment of cattle.

SUMMARY

Three series of experiments are described showing the effects of Dimidium Bromide (1553) against *T. congolense* infection of Zebu cattle.

In the first and second series the high trypanocidal effect of the drug is demonstrated. In the third series, results are described which suggest that the use of the drug may be contributory to certain unpleasant delayed sequelae which have been likened to the syndrome of photosensitisation.

The experiments are discussed and it is concluded that the drug is an effective trypanocide, but that its use is attended by the risk of delayed photosensitisation. This is attributed to a build-up of photodynamic substances in the blood after liver dysfunction has been caused by Dimidium Bromide.

A suitable dose for field use is suggested and a warning regarding the instability of solutions of the drug is given.

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PHENOTHIAZINE FOR THE CONTROL OF HELMINTHIASIS IN LAMBS: MONTHLY DOSING COMPARED WITH A PHENOTHIAZINE-SALT MIXTURE

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INTRODUCTION

In recent years a number of workers, particularly in America, have claimed that self-medication of sheep by allowing them free access to mixtures of phenothiazine and granular salt will control helminthiasis at a harmless level, if treatment commences before the infestation has become acute.

Boughton and Hardy (1941), working in Texas, controlled helminthiasis in weaned lambs with a mixture of one part of phenothiazine to three parts of salt; but oxidation derivatives passed in the faeces caused excessive staining of the fleece. Later workers in America have used smaller proportions of phenothiazine in the mixture and, generally speaking, the method has been reasonably successful in certain districts. The results in Australia, however, have been less promising. The Annual Report of the Council for Scientific and Industrial Research (1944-45) states that, with mixtures of 1 : 15 and 1 : 30 phenothiazine-salt, the daily intake was less than 0·2 g. per head, and Reid (1946) does not recommend the method for treating sheep under Australian conditions.

In this country Harbour, Morgan, Sloan and Rayski (1946), in several experiments in Scotland, found 1 : 14 and 1 : 9 phenothiazine-salt mixtures compared well with standard monthly doses of phenothiazine. Both mixtures depressed the faecal egg-counts and lowered the infection of pasture larvae, while the lambs treated with the 1 : 9 mixtures showed significantly higher weight gains than the untreated controls. Further references to the subject are given by these authors.

The present experiment was planned as an extension of the above trials by Harbour *et al.*, and incorporated groups to test the effect of dosing ewes prior to lambing, a point that had not been investigated adequately in previous work. An experiment of this scope could not be done at the Cooper Field Research Station, and Professor A. N. Worden, of the Department of Animal Health,

* Seconded to the Department of Animal Health, University College of Wales, Aberystwyth, when this work was in progress.

University College of Wales. Aberystwyth, agreed to make available a 23 acre field in a third year ley at Pantyrhuad Farm, St. Clears, Carmarthenshire and to provide staff to assist in the field work involved.

When this work was planned it was realised that field experiments of this type suffer from many disadvantages and arrangements were therefore made for complementary experiments to test the effect of set daily doses of phenothiazine on pure infestations. This work was done at the Cooper Field Research Station, Little Gaddesden, Herts and is reported elsewhere in this journal (Page, 1949).

EXPERIMENTAL MATERIALS AND METHODS

Pasture.—Ebenezer field, situated in the southern upland portion of Pantyrhuad farm, was in the third year of a ley composed of timothy, cocksfoot, Italian and perennial ryegrass, and red and wild white clover at the start of this experiment. It was divided into six plots, each of 3·7 acres. These plots were long and narrow, the shape being necessary to eliminate inequalities in the pasture and each had a collecting pen at one end.

Mr. Trefor Thomas, the Senior Agronomist of the Welsh Plant Breeding Station, kindly surveyed Ebenezer field before the sheep entered the plots and at intervals until the end of the experiment. His report on the botanical analyses of the pasture on these occasions is given in Appendix I.

Test Animals.—The ewes and their lambs were drawn from a flock used in another experiment to test the effect of dosing in-lamb ewes with phenothiazine. They supplied the two main groupings used for the experiment, *i.e.*, one group dosed and one not dosed during pregnancy. Within this framework there were duplicate sub-groupings as follows : (i) 1 : 10 phenothiazine-salt mixture (Plots 1 and 4) ; (ii) Monthly dosing with phenothiazine with free access to plain salt (Plots 2 and 5) ; (iii) Untreated controls with free access to plain salt (Plots 3 and 6).

A total of 17 ewes and 25 lambs was allocated to each plot ; the ewes were all the Improved Welsh Mountain breed, the majority giving pure-bred lambs and the remainder Clun Forest crosses. The lambs were ear-numbered within a few days of birth.

It had been hoped that the experiment would commence when the lambs were only a few weeks old, but difficulties of obtaining fencing materials for enclosing the plots made this impossible. As a result the lambs were three to three and a half months old when they entered the plots and, since prior segregation of the main experimental groupings had not been possible, they had been exposed to a uniform chance of acquiring infection from the pasture. It will be seen that this probably did occur and obscured any effect of the dosing of the ewes.

Treatment.—The phenothiazine-salt mixture was supplied in small weather-proof troughs. Both the untreated controls and the lambs given monthly doses of phenothiazine had free access to plain granular salt in similar covered troughs. The lambs in the groups dosed monthly were given an initial dose of 10 g. of phenothiazine in aqueous suspension, and thereafter 20 g. per head at monthly intervals.

Observation of Results.—At intervals of four weeks, live weights were recorded by weighing on a spring balance and faecal samples were taken

from the rectum on three successive days from all the lambs. A joint 6 g. sample per lamb, made up of three 2 g. samples from three days' collections, was put up for egg-counting by a modified McMaster technique. The essential points of this modified technique (Craufurd-Benson) were (*a*) passing the faeces-brine suspension through a screen of thirty mesh to the inch and (*b*) counting four McMaster cells. In the examination of these samples a straight positive or negative diagnosis was made for *Moniezia* sp. and the nematode ova were counted in two classes, *viz.*, *Nematodirus* sp. and "*Other Species.*"

On each third day of sampling per month, large samples of faeces were taken from as many lambs as possible in each group. Ten grammes from each of these was put up in a bulked culture to test possible inhibition of development by phenothiazine or its derivatives. The counts on which the estimated egg content of these cultures was based included *Strongyloides* eggs as a separate count. The cultures were incubated at 25° C. for 14 days and extracted by the Baermann technique.

The administration of the monthly doses to Groups II and V was made mid-way between the faecal sampling dates and, at the same time, herbage samples were taken by the technique advised by Taylor (1939) for estimating the infestation of larvae on the pastures. These samples were subsequently extracted by the modified method used by Harbour *et al.* (1946); 0·1 per cent. solution of NaOH being used in place of water in the final Baermann extraction to kill the free-living larvae. The larvae were pipetted in streaks on to $1\frac{1}{2} \times 3$-inch slides and counted under a binocular dissecting microscope; doubtful specimens were examined with a high power.

Autopsies.—At the conclusion of the experiment a total of 72 lambs, in two batches of 36 (September and October), were killed at Carmarthen slaughterhouse. By arrangement with the Ministry of Food, the viscera were retained from all these lambs and total worm counts on them were made in the laboratory. The contents of the abomasum and small intestine were each diluted to 2,000 ml., the walls of the organs being carefully examined and scraped; three 10 ml. samples were taken from each suspension after thorough stirring and all the worms were picked out and identified; total worm burdens were estimated from these counts. The contents of the large intestine were washed through a screen of eight mesh to the inch and all the worms were counted. At the same time, various data on carcase weights and measurements were recorded as an index of the condition of the lambs.

RESULTS

Egg Counts

It will be seen that the egg counts for nematodes classed as "Other Species," which includes all the species present (except *Nematodirus*) known to be pathogenic (*i.e.*, mainly *Trichostrongylus* spp. and *Ostertagia* spp.—see Table IV), were similar, at the beginning of the experiment, in all six groups (Table I). This shows that the effect, if any, of dosing the ewes during pregnancy was lost by the time the trial commenced and indicates good randomisation of the test animals. Owing to this initial parity of the main groupings the results are subsequently considered in terms of the means of duplicate pens, regarding the latter as homogeneous samples.

TABLE I

INITIAL OBSERVATIONS OF INDIVIDUAL PLOTS—ILLUSTRATING THE PARITY OF THE MAIN EXPERIMENTAL GROUPINGS

Pre-lambing history	*Plot number*	*Treatment*	*Mean faecal egg count*		*% Infected Monniezia sp.*	*Live weight*	*Pasture larvae per lb. herbage*
			Nematodirus	*Other species*			
Ewes not dosed during pregnancy	1	Phenothiazine-salt	240	228	48	38·5	16
	2	Monthly dosing	300	220	44	39·6	15
	3	Controls	288	193	43	36·4	19
Ewes dosed during pregnancy	4	Phenothiazine-salt	271	367	35	37·2	16
	5	Monthly dosing	278	215	39	36·1	43
	6	Controls	285	250	61	37·0	8

The mean egg count of the groups treated with the phenothiazine-salt mixture showed a slight but consistent fall from the initial figure of 294 e.p.g. to a final level of 115 e.p.g. The groups receiving monthly doses of phenothiazine dropped suddenly from the initial figure of 212·5 e.p.g. to a negligible count of 5 e.p.g. (representing only five positives amongst 50 lambs) after the first treatment and, apart from a slight rise to 101 e.p.g. in September, remained at a very low level throughout the experiment. The untreated controls were consistently higher than their initial level of 225 e.p.g. and reached a peak of 588·7 e.p.g. in August (Table II and Fig. 1).

TABLE II

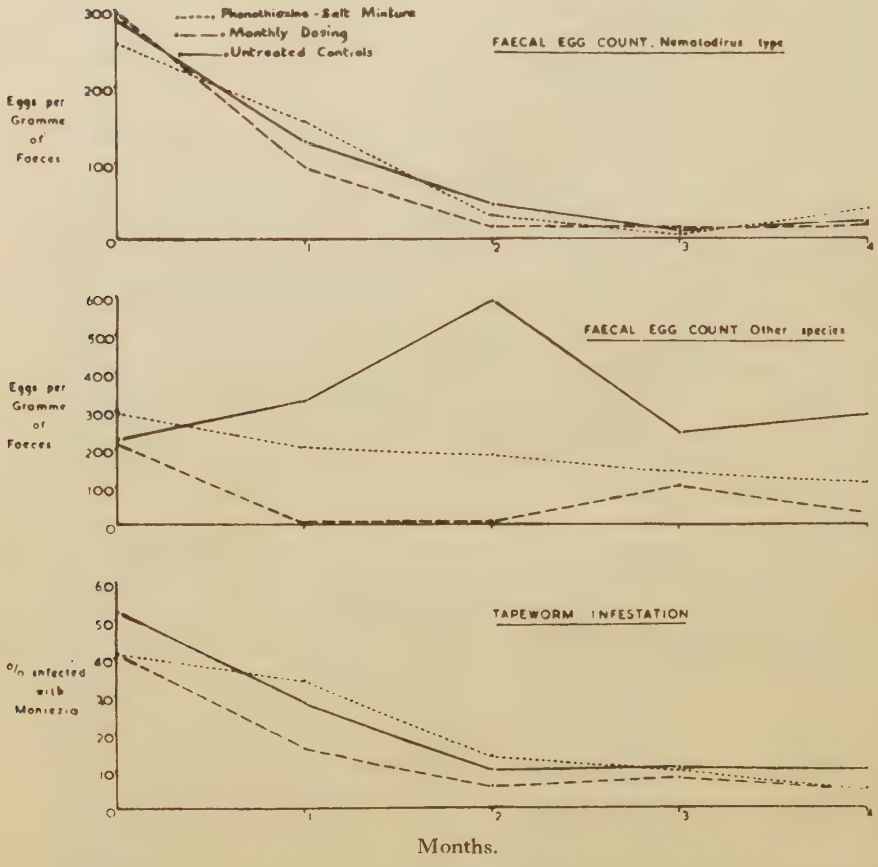
MEAN MONTHLY EGG COUNTS OF DUPLICATE PENS

Species	*Group*	*May-June*	*July*	*Aug.*	*Sept.*	*Oct.*
Nematodirus sp. (eggs per g. of faeces)	Phenothiazine-salt	255·2	154·0	32·3	7·3	40·0
	Monthly dosing	297·9	94·0	16·6	15·9	20·0
	Untreated controls	286·4	127·0	46·8	13·0	21·0
Other species (eggs per g. of faeces	Phenothiazine-salt	294·8	205·0	184·7	139·6	115·0
	Monthly dosing	212·5	5·0	7·5	101·0	32·5
	Untreated controls	225·0	315·0	588·7	243·5	289·5
Moniezia (% infested)	Phenothiazine-salt	41·7	34·0	14·6	10·4	5·0
	Monthly dosing	41·7	16·0	6·4	8·5	5·0
	Untreated controls	52·3	28·0	10·6	10·9	10·5

The egg counts of *Nematodirus* sp. were also approximately the same for all groups at the beginning of the experiment and subsequently fell at the same rate, irrespective of the treatment received (Table II and Fig. 1). By the end of the experiment the mean counts of all groups were at a very low level (20 to 40 e.p.g.), although *post-mortem* examinations revealed substantial infestations of adult *Nematodirus* sp. in all groups. These results confirm earlier evidence

that phenothiazine has no effect on *Nematodirus* sp. (Seghetti and Marsh, 1945 ; Eveleth and Goldsby, 1945 ; Elder, Crisler and Gentry, 1946). This nematode is essentially a parasite of young stock and the uniform fall in these egg counts may be attributed to the development of age immunity in the lambs, which is manifested mainly by a suppression of the egg production of females and possibly also some elimination of adults. (Taylor, 1934.)

Fig. 1.



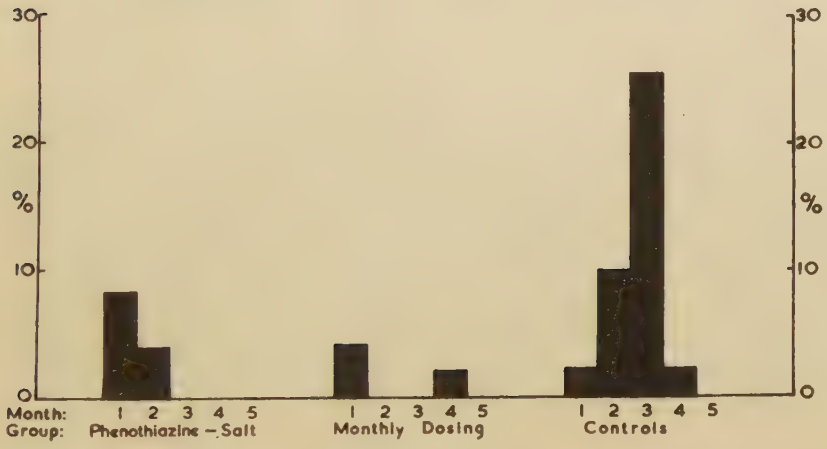
Infections of the tapeworm *Moniezia* were also at about the same level in all groups in May and all fell in the same way as *Nematodirus*. Under the circumstances the initial incidence of *Moniezia* was surprisingly high (40 to 50 per cent.) : the Orebatid mites which act as the intermediate hosts of this tapeworm usually thrive only on a dense mat of humus and herbage such as occurs on old permanent pastures, and on the comparatively young leys at Pantyrhuad that have been reseeded after a rotation, one would

expect only light infections. These results indicate that (*a*) the infections were probably acquired before the lambs were put on the plots, (*b*) phenothiazine is ineffective against *Moniezia* sp. (again confirming earlier work—Reid, 1946; Elder *et al.*, 1946), and (*c*) lambs develop an age and/or acquired immunity which enables them to throw off the infection at an age of some seven to eight months.

The above results deal only with the average level of infection of the groups. From a practical farming point of view the merits of anthelmintic prophylaxis are better assessed in terms of its ability to prevent sub-clinical infections assuming an acute epizootic status. In Figs. 2 and 3 two standards have been taken as an

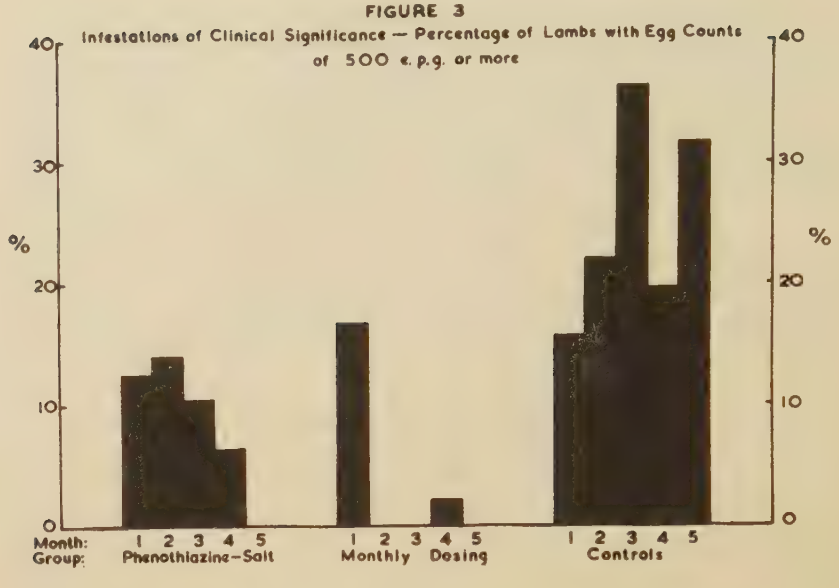
FIGURE 2

Infestations of Clinical Significance — Percentage of Lambs with Egg Counts of 1,000 e.p.g. or more



indication of clinical significance, *i.e.*, 500 e.p.g. and 1,000 e.p.g. Although the former is lower than that generally accepted as a standard, it must be remembered that the infections present in these lambs were mainly *Trichostrongylus* spp. which have a very low rate of egg production. It will be seen from Fig. 3 that at the beginning of the experiment all groups included roughly the same proportion of lambs with egg counts over 500 (c. 12 to 17 per cent.). This proportion fell in the phenothiazine-salt group and, at the end of the experiment, all the lambs receiving the mixture were below this level. The monthly dosing group showed no infections of clinical significance immediately after the first treatment and remained thus throughout the experiment except for a rise to just over 2 per cent. in September.

FIGURE 3



Pasture Larvae

The initial estimations of pasture larvae, made just before the sheep were put on the plots, showed that the pasture infestations were low and reasonably uniform. The phenothiazine-salt plots showed a slightly higher infestation after one month, but subsequently, when the lambs had become accustomed to taking the mixture, fell to a low level and remained thus throughout the experiment. The larvae on the monthly dosing plots rose to a peak of 460 in the first month, then fell and remained at a low level. The untreated control plots showed a similar initial rise and remained high, reaching a peak of 700 larvae per pound of herbage at the end of the experiment in mid-October (Table III and Fig. 4).

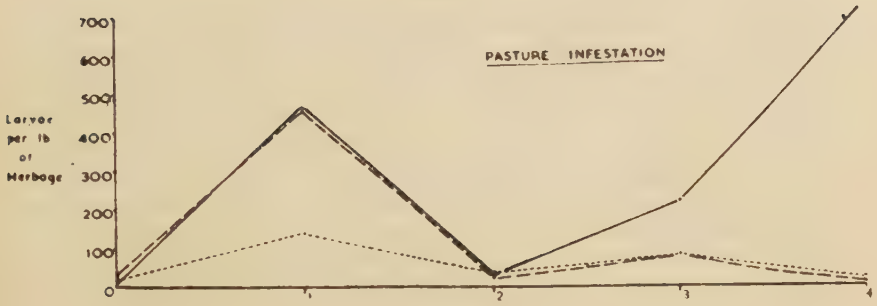
TABLE III

PASTURE INFESTATION. INFECTIVE LARVAE PER LB. OF HERBAGE

Group	*May-June*	*July*	*August*	*September*	*October*
Phenothiazine-salt ...	16	141	42	88	25
Monthly dosing	29	460	26	88	13
Untreated controls ...	14	471	39	213	700

The August counts were low on all the plots. This was probably due to weather conditions at the time the herbage samples were taken ; on this occasion there was a period of bright sunshine following several days of rain, while all the other sampling days were overcast and damp. The low counts for August may, therefore, not be due to any effects of treatment.

FIG. 4.



Inhibition of Larval Development

Attempts were made to confirm the claims of previous workers that the presence of phenothiazine or its derivatives inhibits the development of larvae from eggs voided in the faeces. The results of percentage development to the infective stage were too erratic to permit any conclusions on this score. It is considered that this was due to faults of the culturing and Baermann techniques used for extracting the cultures rather than to variable effects of the treatment. In our opinion this technique is not reliable for any quantitative or comparative work and further experiments on this point should not be attempted until a more satisfactory method has been devised. The most that can be said from the present results is that all the plots, on all occasions, yielded viable infective larvae from faecal cultures. While it is possible that some degree of inhibition of development may have occurred with the phenothiazine-salt treatment, it was never 100 per cent. and varied from time to time. Since the average daily consumption of phenothiazine during this experiment is estimated to have been approximately 0·18 g. per head, this result is in agreement with Shorb and Habermann (1940) who found that a minimum daily intake of 0·5 g. was necessary to ensure complete inhibition of development of *H. contortus* eggs (cf. Page, 1949).

Post-mortem Worm Counts

The results of *post-mortem* helminthological examinations show that the bulk of the infections were *Trichostrongylus* spp. in both the stomach (*T. axei*) and small intestine (*T. vitrinus* and *T. colubriformis*). *Ostertagia circumcincta* was present in small numbers in the abomasum and a few *Cooperia curticei* occurred in the small intestine in most lambs. *Nematodirus filicollis* was present in fairly large infestations in all groups. *Monodontus trigonocephalus*, *Chabertia ovina*, *Oesophagostomum venulosum*, and *Trichuris ovis* also occurred in most of the lambs. No *Haemonchus contortus* were found. This species was encountered in lambs at Pantyrhuad Farm in previous years, but routine dosing of the flock with phenothiazine

had probably eliminated it by the time this experiment started. The total worm burdens show that the controls carried the highest infestations in September and October, the lambs in the monthly dosing groups were lowest and the phenothiazine-salt groups were intermediate. (Table IV.)

TABLE IV

WORM COUNTS AT POST-MORTEM EXAMINATION

Organ	*Species*	*September*			*October*		
		P-S.	*M.D.*	*U.C.*	*P-S.*	*M.D.*	*U.C.*
Abomasum	Ostertagia :						
	Males	38	0	25	0	10	17
	Females	183	25	43	50	70	54
	Trichostrongylus :						
	Males	59	25	46	4	85	62
	Females	125	42	129	63	160	459
Small intestine	Trichostrongylus :						
	Males	204	37	242	106	255	404
	Females	409	122	916	707	620	995
	Nematodirus :						
	Males	79	92	340	77	10	29
	Females	271	454	536	465	15	129
	Cooperia :						
	Males	4	0	38	38	0	4
	Females	84	4	35	110	5	0
	Monodontus :						
	Males	4	0	25	0	0	0
	Females	17	0	25	0	0	0
Large intestine	Oesophagostomum :						
	Males	2	3	4·5	2·4	4·4	6·8
	Females	2·5	2·7	6·2	2·2	2·5	8·6
	Chabertia :						
	Males	0·04	0·04	0·40	0	0	0
	Females	0·1	0	0·6	0·3	0	0·3
	Trichuris :						
	Males	3·7	6·3	6·7	4·7	10·8	7·3
	Females	6·1	7·2	8·4	4·3	12·4	8·5
Total (all species)		1,491	820	2,427	1,634	1,260	2,185

Note.—The above figures each represent the average worm burden of 12 lambs, *i.e.*, two batches of six lambs each from duplicate plots.

These results do not show exact quantitative correlation with some of the other data from this experiment. Generally speaking, the differences in faecal egg count between the controls and the two treated groups were much greater than would be anticipated from the respective worm burdens. It is obvious that both of the treated groups, while better than the controls, still carried substantial infestations. This confirms evidence (Page, 1949) that phenothiazine may suppress egg production without eliminating the infestation of adult worms. In both of the treated groups there was some anthelmintic effect on *Trichostrongylus* spp. (Mean Trichostrongylus spp. counts for September/October: Phenothiazine-salt

834, Monthly Dosing 658 and Controls 1,627): although, as indicated by the results of several workers (C.S.I.R., Aust., 1945 ; Eveleth and Goldsby, 1945 ; Seghetti and Marsh, 1945), the effect was by no means complete. Similar figures for *Ostertagia circumcincta* are less conclusive, although the high infestations of the phenothiazine-salt group in September may have been the result of a period when inadequate amounts of the mixture were being taken.

The rest of the species occurring in the three groups showed differences too slight or too inconsistent to permit any definite conclusions although, on the average, the controls had slightly higher infestations than the two treated groups.

Live Weights

Since the mean live weights of the lambs in all three groups were slightly different at the beginning of the experiment, direct comparisons of live weight increases are possibly not valid, but the increase in weight as a percentage of the initial weight is a fair estimate of any change that may have occurred. (Table V.)

TABLE V

LIVE WEIGHT (LB.). MONTHLY MEANS OF DUPLICATE PENS

Group	*May-June*	*July*	*August*	*September*	*October**
Phenothiazine-salt	38·0	48·3	52·1	54·8	51·4
Monthly dosing	37·9	48·2	53·6	56·7	56·8
Untreated controls	36·7	45·6	51·2	53·4	47·9

* The October figures refer to the residue in each plot after drafting 15 lambs. This drafting introduced a bias in the live weights, differing from group to group, and no direct comparisons can therefore be made between the October live weights and earlier ones.

Compared on this basis the live weight increases from May to September do not appear to differ significantly between the three groups ; if anything the untreated controls made the best weight gains.

Phenothiazine-salt	44·2	per cent. increase
Monthly Dosing	43·9	„ „ „
Untreated Controls	46·2	„ „ „

After the September observations had been made, 15 lambs were drafted from each plot to provide a partial resting period for the grass in readiness for a later experiment ; at the same time the plots were reduced in size to keep the stocking per acre constant. Every effort was made to ensure that the drafted lambs were representative of the whole of each group ; but this was done mainly from the point of view of the helminth infestation and, on a live weight basis, the drafting did in fact introduce a bias in favour of the monthly dosing group.

At first sight, this appears to invalidate any subsequent observations on live weights, but if the live weight increases of the drafted

lambs alone are extracted from the figures for the whole groups, there is an indication that a certain proportion of the lambs in the monthly dosing group was showing a good response to treatment and that these lambs were fortuitously selected for retention at drafting. Comparison of the May to September percentage live weight increases of the drafted and remaining lambs in each group shows that the phenothiazine-salt and control lambs were homogeneous samples and did not include a proportion of lambs achieving significantly greater weight increases comparable with the remaining lambs in the monthly dosing group. Moreover, this response to monthly dosing was taking place even in the early stages of the experiment, although it increased, in comparison with the other groups, during the months of August, September and October, when, with the accumulation of helminth infestation at the end of the summer grazing, the effects of treatment would be expected to show. The above points are illustrated in Table VI.

TABLE VI

PERCENTAGE INCREASE IN LIVE WEIGHTS

Group	*Period*	*Phenothiazine-salt*	*Monthly dosing*	*Controls*
Lambs remaining after drafting:	May-Sept.	46·4	56·1	48·7
	May-Aug.	37·5	47·6	43·8
	Aug.-Oct.	7·1	11·8	3·8
	Sept.-Oct.	0·59	5·8	0·21
	May-Oct.	47·3	65·2	49·1
Lambs drafted in September	May-Sept.	43·7	46·1	46·3

There is thus some evidence of better weight increases in the lambs receiving monthly doses of phenothiazine, although these became significant only in the last two months of the experiment. The figures for August to October increases indicate that the phenothiazine-salt groups were also making better progress than the controls in this respect, but the differences here are not so significant. These results are not in agreement with Harbour *et al.* (1946), who found that lambs receiving a 1 : 9 phenothiazine-salt mixture achieved significantly greater weight gains than untreated controls and from this point of view were better than those given standard monthly doses. During the course of this work the rainfall was fairly heavy and this would very probably account for the low intake of the phenothiazine-salt mixture. Under more favourable weather conditions a higher intake might have been expected to exert a greater influence on weight gains.

The absence of any striking differences in live weight gains is possibly due to factors unrelated to the treatment. It will be seen from Appendix I that the heavy stocking on all the plots (17 ewes and 25 lambs to 3·7 acres) had, by September, produced considerable

changes both in the total keep available and botanical composition of the sward. The two sets of figures given for the botanical analysis are not strictly comparable owing to the different methods of assessment used, but they indicate a definite fall in the quality of the pasture where, owing to lack of topping, a lot of the available herbage was coarse and inedible. It is evident that the pasture at the end of the summer was inadequate for fattening lambs. Another factor which would have the effect of limiting weight gains was the high incidence of sheep maggot fly strike in all groups (c. 80 per cent.) during August.

Dressed Carcase Data

The dressed carcase weights and dressing percentages of lambs killed in September and October are shown in Table VII. Several points of interest emerge from these figures. The average dressing percentage was reasonable in all three groups, ranging from 45 to 48 per cent. for September and from 39 to 44 per cent. for October.

TABLE VII

DRESSED CARCASE DATA

MEANS OF DUPLICATE PLOTS FOR A TOTAL OF 72 LAMBS

Date of killing	*Treatment*	*Live weight*	*Dressed carcase weight*	*Dressing percentage*
September	Phenothiazine-salt	57·9	27·1	46·8
	Monthly dosing	57·7	27·7	48·0
	Untreated controls	61·0	27·5	45·0
October	Phenothiazine-salt	51·9	22·2	42·8
	Monthly dosing	58·3	25·4	43·5
	Untreated controls	46·9	18·1	38·6

Anderson (1943) gives a range of 35 to 65 per cent. for sheep and lambs, with an average dressing percentage of 47·10, which is based on a monthly survey of wholesale slaughters for a ten-year period in the United States (mean live weight 83·31 lb. and a carcase weight of 39·24 lb.). The best dressing percentage for both the September and October killings was given by the monthly dosing group, the worst by the untreated controls. Although this order was the same for both months, the relative percentages were different for the two batches: the phenothiazine-salt group gave a better dressing percentage in October than in September compared with both the monthly dosing and control groups. All three groups gave higher percentages in September than in October. Differences in conformation were too slight to be significant.

Thus, although the difference between the three groups cannot be regarded as highly significant, it is clear that the monthly dosing group gave consistently a bigger carcase and better dressing percentage than the other two groups and, in general, the phenothiazine-

salt group was superior to the controls in these respects. This is a most important result since, from a practical and economic aspect, improved meat production is one of the ultimate criteria of the success of anthelmintic treatment.

DISCUSSION

The results of this experiment do not show that either regular monthly dosing or access to a 1 : 10 phenothiazine-salt mixture were completely successful in the control of low infestations of the smaller stomach and intestinal worms in lambs. It is necessary to point out, however, that even the untreated control group never exhibited the acute epizootic form of helminthiasis which is the cause of most serious losses and which would have been likely to demonstrate more fully the benefits of the treatment. Nevertheless, the general trend of the results is the same from so many aspects that the comparative merits of the treatments can be fairly assessed. The 1 : 10 phenothiazine-salt mixture was inferior to monthly dosing under the conditions of this experiment, but the evidence indicates that it may provide a sufficient measure of control to justify its use, granted an intelligent appreciation of its limitations and the circumstances in which it will give good results.

In its favour, the following points have been brought out by the present results. (1) It produced a slight but continuous reduction in the faecal egg count and had some anthelmintic effect on adult nematodes, as shown by the lower total worm counts compared with the control. (2) It had an immediate and substantial effect on the pasture infestation, which was suppressed to a negligible level throughout the experiment. The phenothiazine-salt mixture was slightly superior to monthly dosing in this respect at the beginning of the experiment, although subsequently there was no significant difference. The reduction in pasture infestation was greater than one would have anticipated from the comparatively slight reduction in the faecal egg count and it is possible that, in spite of the lack of supporting data from faecal cultures, some degree of inhibition of development occurred. (3) Apart from suppressing the mean faecal egg count, the mixture gave a steady reduction in the number of individual cases bearing infestations of a clinical status, assessed on egg counts of 500 and 1,000 e.p.g. respectively. Although sub-clinical infestations of the type that remained are of considerable economic importance, they do not lead to the severe losses often caused by the acute form. (4) The lambs treated with the phenothiazine-salt mixture gave a better dressing percentage than the untreated controls. (5) From a practical point of view self-medication of stock by allowing them free access to mixtures incorporating anthelmintics has certain advantages. It effects great economies in time and labour during the season when this is a vitally important consideration and by keeping infestations to a sub-clinical level prevents scouring ; moreover, it is cheaper than regular monthly dosing.

On the other hand, certain disadvantages inherent in the method must be considered. Unless the sheep under treatment are accustomed to trough feeding it is usually necessary to devote some time to schooling them to take the mixture by penning them in a small area around the trough for a few hours each day. Even then, unless the animals are suffering from a definite salt deficiency, it is unlikely that they will ingest with regularity an adequate amount of the mixture for completely successful results. The most satisfactory results with this method have been obtained in the arid regions of the United States, where the stock have normally to be provided with salt licks. It is probable that this factor was exacerbated by the situation of Pantyrhuad farm in the present experiment since the farm is only about three miles from the sea and the pastures are more likely to contain an excess than a deficiency of salt. It is also impossible to ensure a uniform intake of the mixture either by all the lambs or at different times during the grazing season.

It should be realised that this method is not contemplated as an alternative comparable in efficiency to regular monthly dosing. What is expected of it and should be realised, given the conditions to ensure an adequate intake, is a maintenance of the *status quo,* a reduction of the pasture infestation and at best a slight, but consistent anthelmintic effect. This point has been emphasised several times in the literature (Thorp *et al.,* 1944 ; Hull, 1946). It seems likely that this method will be most useful if it is employed in conjunction with normal phenothiazine treatment. If young lambs can be schooled to take adequate amounts of the mixture from an early age, treatment by drenching need be resorted to only if an occasional outbreak of parasitic gastritis occurs. It is suggested that in-lamb ewes should be dosed as least once with phenothiazine (not within 14 days of expected parturition), and that both ewes and lambs should be given free access to a 1 : 10 phenothiazine-salt mixture from the time the lambs are about four to six weeks old. If clinical symptoms of helminthiasis appear, standard doses of phenothiazine should be given to both ewes and lambs.

SUMMARY

An experiment to compare the merits of a 1 : 10 phenothiazine-salt mixture with standard monthly doses of phenothiazine for the control of helminthiasis in lambs is reported. *Trichostrongylus* (both stomach and intestinal species), *Ostertagia,* and *Nematodirus* were the predominant genera encountered. Small numbers of *Cooperia, Monodontus, Oesophagostomum, Chabertia* and *Trichuris* were also present in most of the lambs.

Monthly doses of phenothiazine (20 g.) were more efficient than the phenothiazine-salt mixture in reducing the mean egg count, the proportion of clinical cases of helminthiasis and the total worm burden.

The phenothiazine-salt mixture was intermediate between the monthly dosing group and the untreated controls with respect to the above points, but was superior to both for control of the pasture infestation although the average daily intake of phenothiazine in this group was slightly less than 0·2 g. per head. There was some evidence that the monthly dosing group achieved the best live weight gains and the controls the worst. At killing, the mean dressing percentages of the three groups were also in the above order.

Neither monthly dosing with phenothiazine nor free access to the phenothiazine-salt mixture gave complete control of low infestations in which *Trichostrongylus* spp. predominated. Both methods of treatment gave partial control, monthly dosing being superior to phenothiazine-salt except for the suppression of pasture infestation.

Although a sufficient intake of the phenothiazine-salt mixture is not likely to be achieved in Great Britain except in regions where there exists a definite salt deficiency, it is considered that this method of treatment may provide a useful compromise if circumstances are such that regular monthly dosing is impossible.

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Appendix I

REPORT BY MR. M. TREVOR THOMAS, ON THE BOTANICAL COMPOSITION OF EBENEZER FIELD, PANTYRHUAD FARM, SUMMER, 1946

	Percentage contribution	
	*May**	*September†*
Lolium perenne (Perennial Ryegrass)...	28	34
Dactylis glomerata (Cocksfoot) ...	23	12
Lolium italicum (Italian Ryegrass) ...	3	Trace
Phleum pratense (Timothy)	10	2
Trifolium pratense (Red Clover) ...	14	2
Trifolium perenne (White Clover) ...	2	14
Miscellaneous herbs (unsown) ...	5	5
Other grasses (unsown)	10	19
Inert material	5	—
Bare ground	0	12
	100	100

* Based on separations of representative samples of "air dried" herbage. An analysis based on relative contribution by weight.

† Based on estimations from reading *in situ* of "ground covered" by the various species. There was not sufficient herbage for cuts for productivity analysis. On a productivity basis, Cocksfoot would be higher. The decrease in Italian Ryegrass and Red Clover as relatively short-lived species, would be expected, and Timothy would appear to have suffered from the hard grazing.

Chief Miscellaneous Herbs

Ranunculus spp. (Buttercup); *Carduus* spp. (Thistles); *Plantago* spp. (Plantains); *Rumex* spp. (Docks); *Cerastium* spp. (Mouse-Eared Chickweed); *Hypochaeris* spp. (Catsear).

Chief Unsown Grasses

Holcus lanatus (Yorkshire Fog); *Agrostis* spp. (Bent); *Poa trivialis* (Rough-Stalked Meadow Grass); *Poa annua* (Annual Meadow Grass); *Anthscanthun* sp. (Sweet Vernal Grass).

The management would assist the spread of Wild White Clover. *Agrostis* would appear to have increased, but, in any case, this plant would not have shown to advantage in early May on a production basis.

The data indicate only slight botanical differences between the various plots; both Bent and Yorkshire Fog seem to be rather more prevalent on Plots 4, 5 and 6.

The history of the field is as follows: 1941, Grass; 1942, Mustard; 1943, Wheat; 1944, Reseeded direct; 1945, Hay; 1946, Grazing.

THE ANTHELMINTIC EFFECT OF PHENOTHIAZINE ADMINISTERED IN SMALL DAILY DOSES TO LAMBS BEARING HEAVY INFESTATIONS OF *HAEMONCHUS CONTORTUS*

By

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INTRODUCTION

Phenothiazine-salt mixtures have been used with some success in certain areas of America for the treatment of sheep infected with parasitic nematodes. An average daily consumption of 0·25 to 0·5 g. phenothiazine per head, if achieved, was found to be effective in depressing the worm egg-count and the sheep showed significant weight gains over those receiving salt alone. It has been recorded (Shorb and Habermann, 1940 ; Britton, Miller and Cameron, 1942 ; and others) that small daily doses of phenothiazine resulted in the inhibition of larval development in the faeces of sheep.

Harbour, Morgan, Sloan and Rayski (1946), in trials conducted in S.E. Scotland during 1943-45, found that phenothiazine-salt mixtures were successful in reducing the level of clinical parasitism and that lambs receiving phenothiazine-salt mixtures showed, in the main, significantly greater weight gains than the control groups. The method was not, however, as efficient as regular monthly dosing. The daily intake of phenothiazine per head in these trials was about 0·25 g.

Experiments were planned in 1946 to test certain aspects of this method of anthelmintic treatment. Controlled trials on the administration of small daily doses of phenothiazine to artificially infected lambs kept in yards were to be done at the Cooper Field Research Station, Little Gaddesden, Herts, and these form the subject of this paper ; while a comparison of the merits of routine monthly dosing and self-medication with phenothiazine-salt mixtures under normal field conditions was to be done in Wales. The results of the latter experiment are reported elsewhere in this journal (Pollard, Owen and Evans, 1949).

The trials here reported were designed as controlled experiments using lambs bearing heavy infestations of *Haemonchus contortus*. Pure infestations of the species were used to observe the effect of small daily doses of 0·25 and 0·5 g. phenothiazine on faecal egg-counts, worm population, effect on larval development in the faeces and body weights. Previous workers have found that lambs having free access to phenothiazine-salt mixtures readily took from 0·25 to 0·5 g. phenothiazine daily per head. However, as the daily intake of phenothiazine under these conditions was assessed on the *total amount of the mixture consumed by the group of lambs under treatment over a prescribed period of time, the individual lamb intake can only be an approximate figure of daily consumption.*

In the experiments here reported, to obviate any discrepancy

in individual lamb intake and because of the small size of the dose, the drug was administered in gelatine capsules daily *to each lamb* undergoing treatment.

EXPERIMENTAL MATERIALS AND METHODS

Thirty-five lambs, aged from two to three months, the progeny of Suffolk rams and Scottish Greyface, Half-bred or Hampshire ewes were divided into four groups of five lambs each, and two control groups of five and 10 lambs respectively. The groups had approximately equal mean body weights at the beginning of the trial. Duplicate faecal egg-counts were carried out on every lamb before its inclusion in the trial: as all counts were negative it can be safely assumed that the lambs were relatively worm-free before artificial infestation.

The following were the methods of treatment for each group of lambs:—

Group 1. Five sheep. 0·25 g. phenothiazine daily for 14 days before and 70 days after artificial infestation.

Group 2. Five sheep. 0·5 g. phenothiazine daily for 14 days before and 70 days after artificial infestation.

Group 3. Five sheep. 0·25 g. phenothiazine daily from the 42nd to the 70th day after artificial infestation.

Group 4. Five sheep. 0·5 g. phenothiazine daily from the 42nd to the 70th day after artificial infestation.

Group 5. Ten sheep. Infected, undosed control group.

Group 6. Five sheep. Uninfected, undosed control group.

The period of 42 days after infestation was selected in conformity with the normal egg-count curve of an infection initiated by a single massive dose of larvae based on observations made by Craufurd-Benson (1940).

Infestation of Groups 1 to 5 was carried out on June 24th, 1946, 5,000 third stage infective larvae being administered to each lamb. Administration of such massive doses of larvae resulted in the building up of infestations of a magnitude not normally found in young lambs, but although many untreated lambs showed symptoms of acute parasitic gastritis, no deaths occurred as a result of the extremely heavy worm burden. Some deaths, due to pneumonia of an undetermined cause, did, however, occur in Groups 1, 2 and 6 immediately after the start of the experiment.

All the six groups were kept in a concrete yard which was washed and swept daily to reduce the risk of accidental infection. Ideally each group should have been isolated, but owing to the shortage of suitable yards this was not possible. By concentrating all infected groups into one yard, the risk of re-infection remained approximately equal for each group. The lambs were trough-fed on a balanced ration of roots, kale or tares, hay and concentrates. Fortnightly weighings of all the test sheep were recorded.

Five gramme samples of faeces from each lamb were taken at weekly intervals, and samples from lambs in each group were bulked together and cultured in kilner-type jars for ten days. Extraction of the larvae was made by the Baermann technique, the number of larvae per gramme of faeces being assessed on the total number of larvae recovered.

Administration of the 0·25 and 0·5 g. doses of pure phenothiazine was made in gelatine capsules with the aid of improvised balling-guns. The drug was given daily to each sheep under treatment, including the day of artificial infestation with larvae in Groups 1 and 2 (two hours before infestation). The method of administration of the drug was found to be very efficient, the lambs swallowing the capsules easily; in a few isolated instances the capsules were broken in the mouth but little phenothiazine was lost,

In general the method ensured an accurate dose being given in a form comparable to phenothiazine-salt lick medication.

Faeces samples were taken from each lamb at intervals during the course of the experiment. Faecal egg-counts were then effected using a 2-g. sample. The technique used was a modification of the standard McMaster technique and included (*a*) passing the faeces-brine suspension through a thirty mesh to the inch screen to remove coarse detritus, and (*b*) counting four McMaster cells instead of three. *Post-mortem* examinations were made on two lambs in each group on the 70th day after artificial infestation. Counts were made of all adult worms present in the fourth stomach and intestines of the lambs, or if the number of worms present was too great, by counts of the number of worms present in a measured quantity of stomach or intestinal contents.

EXPERIMENTAL RESULTS

Faecal Egg-Counts

A summary of the egg-counts for each group is given in Tables I, II and III and these data are also shown graphically in Fig. 1. Dosing in Groups I and II was commenced 14 days before the test infestation and continued daily until 70 days later.

Group I. A significant depression of the egg-count compared with the infested controls was afforded by the medication (see Fig. 1), but the reduction in the egg-count was not so marked as in Group II receiving 0·5 g. phenothiazine. The group mean egg-count fluctuated between 1,000 and 3,000 eggs per gramme. Clinical significance is usually attributed to counts of this magnitude in natural infestations in the field. Under the conditions of these experiments, a daily intake of 0·25 g. phenothiazine did not give sufficient protection against the establishment of a possibly lethal infestation after intake of a massive dose of infective larvae.

Group II. An almost complete suppression of the egg-count occurred as a result of the treatment. It will be seen from Fig. 1 that at all times during the course of the experiment the mean egg-count was below that of the uninfested undosed control Group VI. The very low counts recorded in the uninfected Group VI indicate that infestation from ground contamination by larvae was negligible.

Group III. Although the administration of 0·25 g. phenothiazine to lambs bearing established helminth infestations considerably reduced the egg-counts, the level remained high, fluctuating between 3,000 and 6,000 eggs per gramme during the treatment. Under normal field conditions such a count would be an indication of excessive parasitism, but it is unlikely that an initial infestation of such magnitude would occur in a non-immune animal under natural conditions. Taylor (1944) estimates that the daily intake of larvae on heavily infested sheep pasture may be 8,000 larvae per head per day. The main difference between experimental and field conditions is that the larval intake in the latter is a more gradual process, permitting the development of an acquired immunity. It can, therefore, be assumed that the daily administration of 0·25 g. phenothiazine to lambs bearing light-moderate helminth infestations might result in a satisfactory reduction in the egg-count.

TABLE I

DOSING COMMENCING PRIOR TO INFESTATION

Group	*Phenothiazine Dose gm.*	*Mean Egg-Counts (E.P.G.) Days after infestation*																		
		21	22	23	25	30	35	37	39	43	44	45	46	47	51	54	58	61	65	68	70
1	0·25	1083	1700	1683	1683	2250	2053	1483	1383	2600	1367	1400	1717	1933	3050	2467	2267	1283	2100	1633	1750
2	0·5	12·5	12·5	0	0	37·5	12·5	0	0	12·5	12·5	12·5	25	0	0	25	0	12·5	0	0	0

TABLE II

DOSING COMMENCING SUBSEQUENT TO INFESTATION

Group	*Phenothiazine Dose gm.*	*Mean Egg-counts (E.P.G.) Days after infestation*																		
		21	25	30	35	37	39	44	49	50	51	52	53	56	58	60	63	65	67	70
3	0·25	6120	6660	8900	9280	9490	8180	4840	3770	3930	6060	3280	3250	3480	4300	4550	5420	3380	2470	2830
4	0·5	5030	5770	8400	10530	10050	8300	2600	1760	1530	1590	1800	1410	340	410	110	400	720	360	450

Dosing in Groups 3 and 4 was commenced on 42nd day after the test infestation and continued until the 70th day.

TABLE III

CONTROL GROUPS. GROUP 5 INFESTED, UNDOSED (10 SHEEP); GROUP 6 UNINFESTED, UNDOSED (5 SHEEP)

Group	*Phenothiazine Dose gm.*	*Mean Egg-counts (E.P.G.) Days after infestation*													
		21	25	30	35	37	39	44	50	51	56	58	63	65	70
5	Nil	7280	7145	11725	15035	13075	11205	12210	10190	14430	10825	11915	13135	9365	6695
6	Nil	50	20	190	170	100	50	100	—	50	37·5	87·5	50	12·5	87·5

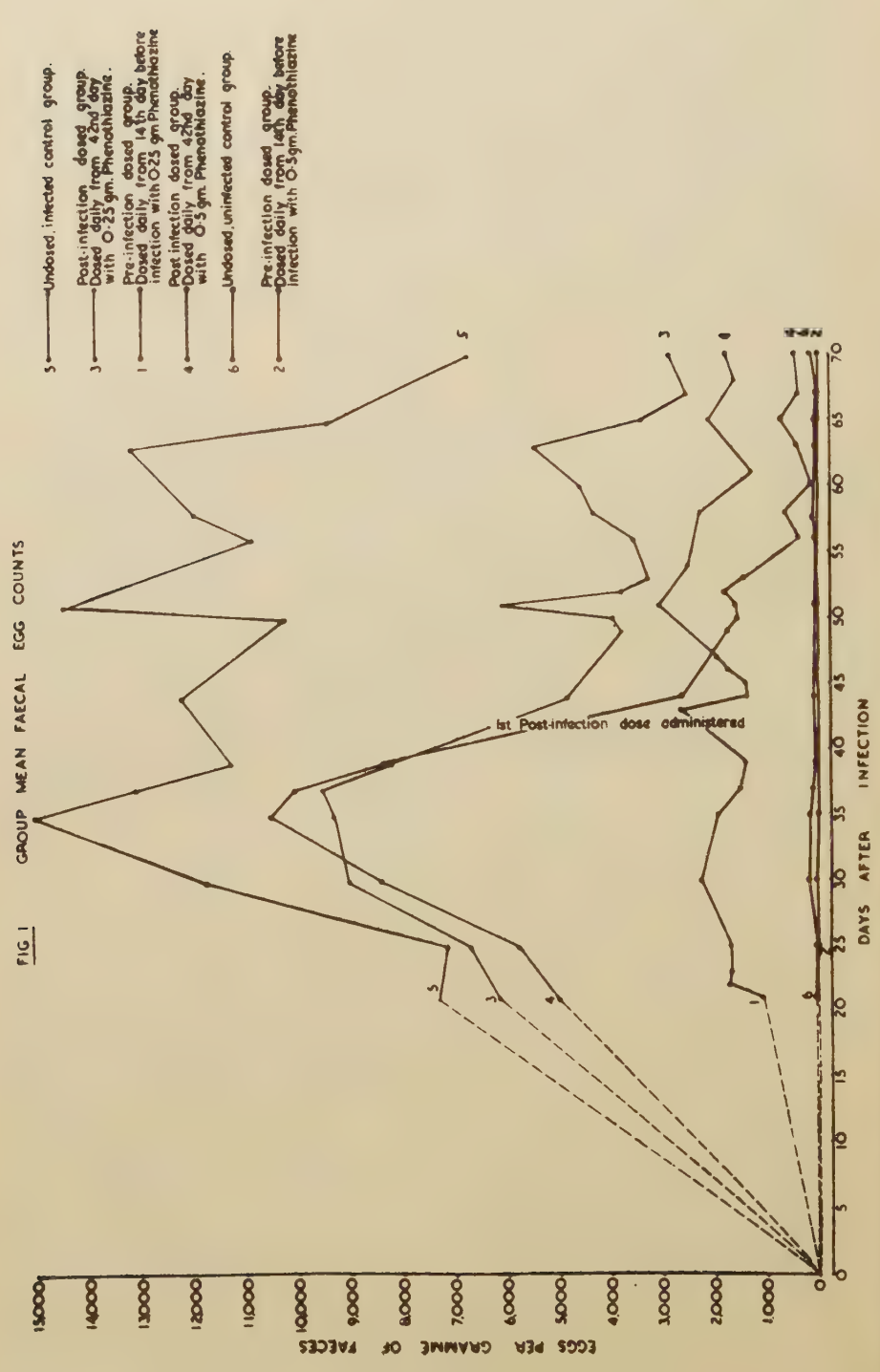

5 Undosed infected control group.
3 Post-infection dosed group. Dosed daily from 42nd day with 0·25 gm. Phenothiazine.
1 Pre-infection dosed group. Dosed daily from 14th day before infection with 0·25 gm Phenothiazine
4 Post infection dosed group. Dosed daily from 42nd day with 0·5 gm. Phenothiazine.
6 Undosed uninfected control group.
2 Pre-infection dosed group. Dosed daily from 14th day before infection with 0·5gm Phenothiazine
1st Post-infection dose administered
EGGS PER GRAMME OF FAECES
DAYS AFTER INFECTION

FIG 1 GROUP MEAN FAECAL EGG COUNTS

Group IV. A marked reduction in the egg-count occurred as a result of the treatment. Complete suppression of the egg-count was not obtained, counts in the region of 300 eggs per gramme being recorded in the 14 days prior to the end of the experiment. These counts, expressed as a percentage of the original infestation, show an approximate reduction in the egg-count of 97 per cent. which, taking into account the intensity of the established infestation, is extremely satisfactory.

Group V. Group mean faecal egg-counts of from 7,000 to 15,000 eggs per gramme were recorded. Throughout most of the experimental period the count followed the normal egg-count curve for a pure infestation initiated by a single massive dose of larvae. The decline in the egg-count toward the end of the experiment is an indication that the ten lambs which constituted the group were, by this time, acquiring some immunity. In view of this decline in the egg-output of the control group it was decided to end the experiment.

Group VI. This group, which was included in the trials as a control group to indicate the degree of contamination from the yard, showed some evidence of low helminth infection throughout the course of the experiment. Group mean egg-counts ranging from 13 to 190 eggs per gramme were recorded. It is evident that although some natural infestation took place as a result of the close-penning of the lambs in the yard, it was not great and did not materially affect the outcome of the experiment.

Post-mortem Examinations

The results of *post-mortem* examinations carried out on two lambs from each group are given in Table IV.

It will be seen that the total worm burdens of the lambs confirmed the general picture given by the egg-counts. There appears to be some indication that small daily doses of 0·5 g. phenothiazine are more toxic to adult female worms than to the males. The ratio of males to females in the pre-infestation dosed group of 4 : 1 is abnormal, but no definite conclusions can be drawn from these figures as the numbers of lambs slaughtered from each group are too small. Fig. 2 shows the group mean worm burdens of all the groups plotted against the last egg-counts recorded from the lambs slaughtered. There appears to be a considerable inhibition of egg-production in the phenothiazine treated groups, whilst the control groups appear normal.

Inhibition of Larval Development in the Faeces

Under the conditions of these experiments there was no evidence to show that the daily administration of 0·25 or 0·5 g. phenothiazine inhibited larval development in the faeces. The Baermann extraction technique used for the recovery of larvae, used in conjunction with our normal faecal culture methods, is not suitable for

trials of this nature. The method is by no means quantitative owing to a fluctuating larval recovery rate, and is only suitable for general larval extraction from stock cultures.

TABLE IV

POST-MORTEM WORM COUNTS

Group	Lamb number	Fourth stomach				Small intestine			
		Species	Male	Female	Total	Species	Male	Female	Total
1	77	*H. contortus*	450	250	700†	*Nematodirus filicollis*	0	2	2
						Trichostrongylus spp.	0	1	1
	81	*H. contortus*	50	50	100*	*Cooperia oncophora*	0	1	1
2	41	*H. contortus*	6	1	7	*Nematodirus filicollis*	7	6	13
						Cooperia oncophora	2	3	5
	52	*H. contortus*	3	1	4	*Nematodirus filicollis*	1	0	1
3	73	*H. contortus*	350	400	750*	*H. contortus*	95	114	209
						Nematodirus filicollis	0	2	2
		Ostertagia circumcincta	0	25	25	*Ostertagia circumcincta*	0	2	2
						Trichostrongylus spp.	0	1	1
	92	*H. contortus*	650	825	1,475*	*H. contortus*	6	8	14
						Nematodirus filicollis	4	4	8
						Cooperia oncophora	0	1	1
4	33	*H. contortus*	254	107	361	*Cooperia oncophora*	0	1	1
	43	*H. contortus*	550	300	850*	*Nematodirus filicollis*	1	5	6
						Cooperia oncophora	1	2	3
						Trichostrongylus spp.	1	1	2
5	94	*H. contortus*	900	850	1,750*	*Nematodirus filicollis*	1	3	4
						Cooperia oncophora	0	2	2
						Trichostrongylus spp.	0	5	5
	98	*H. contortus*	450	850	1,300†	*Nematodirus filicollis*	1	1	2
						Cooperia oncophera	0	2	2
6	57	*H. contortus*	0	6	6	*Nematodirus filicollis*	1	3	4
						Cooperia oncophora	0	2	2
	71	*H. contortus*	0	2	2	*Nematodirus filicollis*	0	2	2
						Cooperia oncophora	0	1	1

* Estimation of numbers by total count of adult worms present in 4 × 20 c.c. samples of a 1-litre dilution of stomach and intestinal contents.

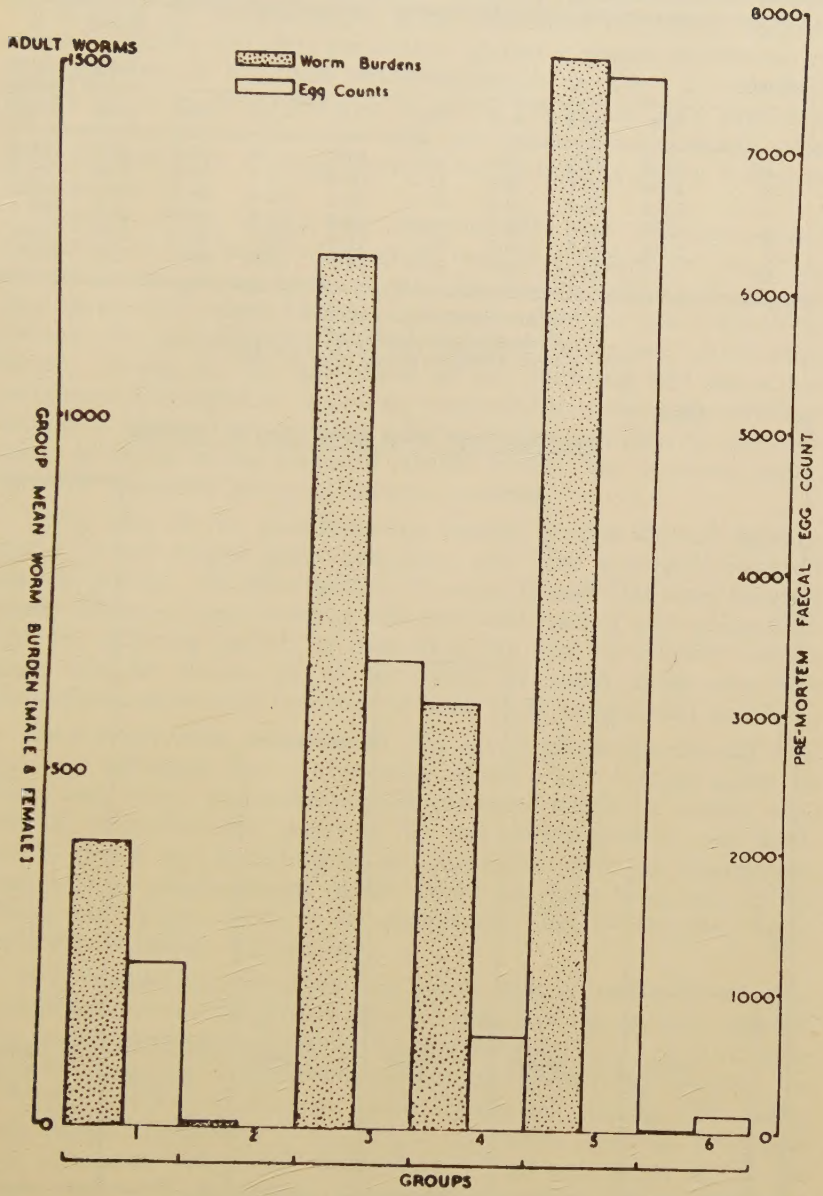
† As above, on a 2-litre dilution. *N.B.—Trichuris ovis* present in all groups.

Generally the results obtained from faecal cultures indicate that although the numbers of larvae present in the faeces were considerably reduced by the administration of small doses of phenothiazine, this decline was probably due to the reduction in adult female worms and therefore egg-output, rather than to any inhibitory effect of the drug on the eggs or larvae in the faeces.

Effect on Weight Gains

The results of fortnightly weighings of all the groups of lambs are given in Table V. Fig. 3 shows the group mean total weight increases for the period of the experiment. Only two Groups, II and III showed significantly higher weight increases than the

FIG 2. Worm Burdens & Faecal Egg Counts



infected untreated control Group V. The total weight gained by all groups was low, but it should be realised that (*a*) the lambs were closely penned in a concrete yard and were subjected to daily handling, (*b*) the majority of the lambs were heavily infected with worms, and (*c*) they were weaned abnormally early in order to reduce the possibility of ewe-to-lamb infection. These factors are not conducive to a rapid and sustained gain of body-weight in lambs

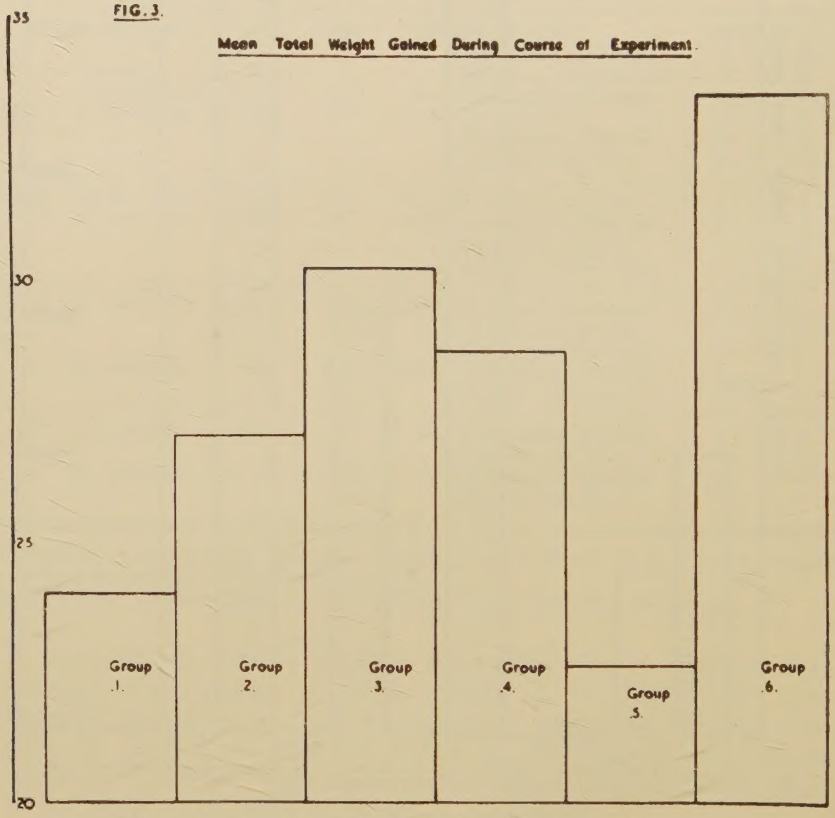
TABLE V

RESULTS OF FORTNIGHTLY WEIGHINGS. GROUP MEAN LIVE BODY WEIGHT (LB.)

Group	*Dates*							*Group mean total weight gain*	*% Increase over initial weight*
	6.6	20.6	4.7	18.7	1.8	15.8	29.8		
1*	32·3	33·7	36·0	41·3	45·0	51·0	56·3	24·0	74·3
2†	31·0	31·5	36·8	41·5	45·3	51·8	58·0	27·0	90·3
3	34·2	38·6	43·2	48·4	52·4	57·8	64·4	30·2	88·3
4	39·6	42·6	47·4	52·4	56·8	61·6	68·2	28·6	72·3
5	29·4	31·6	35·7	39·1	41·3	46·8	52·0	22·6	76·8
6‡	30·0	33·5	39·5	44·8	49·3	55·8	63·5	33·5	111·2

* Three sheep in group after 12.7.46.
† Four sheep in group after 11.7.46.
‡ Four sheep in group after 8.8.46.

FIG. 3.



There did not appear to be any significant relationship between intensity of helminth population and increase in body weights of the lambs in the dosed groups.

DISCUSSION

The results of these experiments show that a daily intake of 0·25 g. phenothiazine per head will reduce the level of an acute

helminth infestation in young lambs, but should the hazard of continuous re-infestation by larvae be great, such treatment might not prevent the establishment of an infestation of clinical significance. Virtually complete protection against a single infestation by large numbers of helminth larvae is afforded by the daily administration of 0·5 g. phenothiazine to young lambs. A daily intake of 0·5 g. phenothiazine per head will also result in a practically complete depression of the egg-count in lambs bearing heavy helminth infestations.

Gordon (1940) found that phenothiazine was effective against immature *H. contortus* when given once at a dosage rate of 0·6 g. phenothiazine per kg. body weight. The results in Group II suggest that small daily doses of 0·5 g. phenothiazine per lamb effectively prevent the establishment and development of ingested third stage larvae. Owing to the deficiencies of the culturing and extraction techniques employed, it cannot be concluded that the daily administration of either 0·25 or 0·5 g. phenothiazine inhibits the development of larvae in the faeces of treated lambs. An accurate assessment of this point was not, however, possible.

Only two of the phenothiazine treated groups showed significantly greater weight gains compared with the untreated infected control group. As these groups (Groups II and III) were bearing vastly different worm burdens and had been treated with phenothiazine for differing periods of time, it is felt that no definite conclusions can be drawn as to the effect of small daily doses of phenothiazine on such increases in lamb body weight that occurred.

This experiment, reviewed in relation to phenothiazine-salt lick therapy, indicates that in countries where a salt hunger exists in sheep and where phenothiazine-salt mixtures would be readily consumed, phenothiazine, taken at the rate of 0·5 g. daily per head, would give a real anthelmintic and protective effect. It is apparent that 0·25 g. phenothiazine daily would be more uncertain in its effect, but might possibly be reasonably effective against light-moderate helminth infestations.

It is stressed that the trials deal purely with *H. contortus* whereas consideration must be given to the prevalence of the smaller stomach and intestinal worms (especially *Trichostrongyles* spp. and *Ostertagia* spp.) in sheep kept under conditions of normal farming practice. In a further trial conducted at Little Gaddesden during 1946 using pure initial infestations of *Ostertagia* spp. (with identical groupings and treatment with small daily doses of phenothiazine as in the trial with *H. contortus*), comparative results were obtained. Unfortunately, contamination of the initial *Ostertagia* infestation by *H. contortus* occurred some time during the trial and at *post-mortem H. contortus* were predominant. The effect on the faecal egg-counts of this contamination could not be assessed, as there is a wide variation in the egg-output per female between the species. All conclusions drawn from this series of experiments could only,

therefore, be based on the assumption that the two species were present in approximately equal numbers. As the results from the mixed *H. contortus-Ostertagia* spp. trial served only to confirm within very close limits the results obtained with *H. contortus* alone, it was felt that their inclusion in this paper would lead to confusion, and they have therefore been omitted.

SUMMARY

Experiments are described to determine the anthelmintic efficiency of small doses of phenothiazine, administered daily to lambs bearing pure infestations of *Haemonchus contortus*.

The drug was administered in gelatine capsules at a dosage rate of either 0·25 or 0·5 g. daily to lambs (*a*) for 14 days before, and for 70 days after artificial infestation with massive doses of helminth larvae, and (*b*) from the 42nd to the 70th day after artificial infestation, *i.e.*, when the latter was established.

Daily doses of 0·25 g. reduced the level of established helminth infestations, but did not reduce the degree of parasitism below that of a clinical infestation.

A daily intake of 0.5 g. phenothiazine had a marked anthelmintic effect on lambs bearing heavy infestations of *H. contortus*. Prior medication by daily doses of 0·5 g. phenothiazine protected non-immune lambs from a single massive dose of larvae. Such doses should protect lambs from re-infestation from natural causes, assuming that the degree of natural infestation is not too high.

Inhibition of larval development by small daily doses of phenothiazine was not evident, but could not be excluded owing to the deficiences of the technique used.

ACKNOWLEDGMENTS

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